# EVALUATION OF ROOT'S ARCHITECTURE, GROWTH PERFORMANCE AND FRUITING OF HONEYCRISP™ APPLE SCION GRAFTED ON 8 ROOTSTOCKS IN RESPONSE TO SOIL AND SOLUTION'S PH USING FIELD, AEROPONICS, AND MINIRHIZOTRON GROWING SYSTEMS.

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Ali Saleh Abdullah Al Farqani

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# EVALUATION OF ROOT'S ARCHITECTURE, GROWTH PERFORMANCE AND FRUITING OF HONEYCRISP™ APPLE SCION GRAFTED ON 8 ROOTSTOCKS IN RESPONSE TO SOIL AND SOLUTION'S PH USING FIELD, AEROPONICS, AND MINIRHIZOTRON GROWING SYSTEMS

Ali Saleh Abdullah Al Farqani, Ph. D.

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Apple (*Malus* × *domestica* Borkh.) trees are not always grown in a favorable soil condition. One of the soil and solution properties that influences apple tree growth and fruit quality is pH due to its effects on nutrient uptake affecting the tree's performance. However, the use of suitable rootstocks represents a sustainable solution to alleviate site challenges and unfavorable growing conditions. In this study, apple rootstocks were evaluated under field and greenhouse environments for root distribution, growth performance, and fruit quality. Eight rootstocks (G.11, G.41, G.935, G.202, G.214, M.9T337, B.9, and M.26EMLA) were tested in response to a range of soil pH's (5.0, 6.5 and 8.0) using a pot-in-pot system. In addition, root development and distribution of four of the rootstocks (G 214, G.41, G.890 and M.9) were evaluated in response to the same soil pH's listed above in a minrhizotron system. While another four rootstocks (G.210, G.214, G.41, G.890) were monitored for root architecture and turnover in response to a range of solution pH's (5.5, 6.5 and 8.0) in an aeroponics system.

The pot study showed that soil pH caused no significant difference in trunk cross-sectional area but soil pH treatment did affect fruit peel nutrient concentration P,

Ca, Mg, Fe. S, B, and Zn. TCSA increase was affected by rootstock with the maximum increase with G.935 and the lowest TCSA increase was with B.9. Leaf nutrient analysis showed higher values of K, Ca, Mg, S Fe and Mn at low pH. However, higher P and Zn were found at high pH. However, no significant difference was found in total soluble solids %, fruit's firmness, number of fruit, bitter-pit incident percentage. The highest fruit per tree was found on G.41 and the lowest bitter pit % was reported in G.935. G.11 had the largest fruit size, weight, and length while G.935 had the best red skin color. All fruit maturity parameters showed a significant difference due to soil pH treatments. However the best values of fruit weight, size, and length were found at high pH.

The aeroponics study showed no difference in root architecture parameters among the four Geneva® rootstocks. However, when each rootstock was investigated individually, G.210 was found to have higher root parameters values. The solution pH was found to affect significantly all root parameters measurements. Some parameters showed doubled or tripled improvement at pH 8.0.

Results from the minirhizotron experiment showed no significant difference in all root parameters among the four apple rootstocks we evaluated. Among the soil pH's, significant differences were found in the root count, total root length, and the total root area. At soil pH 5.0, the root count, root length, root volume, and root area were higher than at the other soil pH treatments, while the average root diameter, average root length, average root area, and the average root volume were higher at pH 8.0.

Rootstocks evaluation under various soil and solution pHs is important to assist in selecting the best adapted apple rootstocks for non-optimum soil conditions and for providing proper fertilizers recommendations.

# BIOGRAPHICAL SKETCH

Ali was born in the Sultanate of Oman, a peaceful corner in the Middle East mostly known for growing arid and tropical fruit trees. Surprisingly, there is an elevated mountain area where growers are planting apples and other temperate fruit trees in a challenging environment. He received his bachelor's degree of science from the Sultan Qaboos University in Oman with a major in plant agronomy and horticulture and a minor in plant pathology and entomology. He also received his Master of Science from the same university in Phytotechnology and Pathology. He then joined a research agricultural station where temperate fruit trees are grown and tested. Ali is married and has four children.

***To My Beloved Wife, Farida Al Mamari***

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# CHAPTER ONE

# LITERATURE REVIEW

## 1. The origin and evolution of Apple trees

### 1.1 Apple origin

Apple (*Malus* × *domestica* Brorkh) is believed to be originated in Central Asia, precisely the Almaty (Alma Ata) region in Kazakhstan (Morgan and Richards, 2002). It was found that *M. sieversii* is the prime progenitor and *M. sylvestris* is a main secondary contributor of the domesticated apples (Cornille et al., 2012) along with other progenitor species which include *Malus sieversii* M. Roem, *Malus orientalis* Uglitzk., and *Malus sylvestris* (L.) (Gross et al., 2014). *Malus sieversii* from the Tien Shan mountain region located between western China border and Kyrgyzstan. The region bisects the ancient territory of Turkistan to the edge of the Caspian Sea (Harris et al., 2002). A study using population genetic methods with microsatellite markers or short tandem repeats (STR) have verified that wild-to-crop gene flow is a potential reason for the evolutionary history of the domesticated apple (Peace et al., 2019). It was believed that fruits of the wild relatives of the domesticated apple originated in the Mideast Europe and then were often collected by Neolithic and Bronze Age farmers. However, it is still a mystery when the larger and sweet apple went from central Asia to the West. This could be due to disagreement in referencing the description by the early Sumerian literature that may refer to the indigenous small and bitter fruits species, *Malus orientalis* (Janick, 2015a). However, there is conclusive proof for noteworthy genomic inheritance from *M. orientalis* and *M. sylvestris* as the apple trees moved through centuries to Europe (Cornille et al., 2014, 2012).

### 1.2 Ancient history era

Apple trees and fruits were mentioned in the Sumerian cuneiform when referring to the Garden of Apples in about 1900 BCE (Vanstiphout, 2000). The Hittites who established their empire in Anatolia (Turkey) during 1700 to 1200BC also described a location with about 40 apple trees. By the 1st millennium, BC apple was already part of western agriculture. Even the greatest Greek poets, Homer, talked about the Garden of the Apple of the king Alcinöus, the king of the Phaeacians during the 8th or 9th century BC (Janick, 2015b).

Due to difficulties in propagating apples by cutting, it might be logical to consider that seed dissemination was a possible explanation of how apple trees were grown away from its origin. The merchandise trade route from Central Asia (Silk Road) passing through Bukhara and Samarkand to Persia might carry apple's seeds intentionally or unintentionally (Janick, 2015a; Luby, 2003; Way et al., 1991). Along the route journey, travelers used animals for transportation and seed germination might be facilitated by animals dropping (Juniper et al., 1998).

Propagation through root suckers and grafting were documented as early as 3800 years ago (Harris et al., 2002). At that time, Persia was the supply hub of many fruits from Central Asia and China and by any mean of foldaway, it reached Greece. Around 300 BC the Alexander the Great conquered Persia and the cultivation of fruits was disseminated through the Greek merchandise and growers (Luby, 2003). The Romans then experimented and applied many techniques including grafting, pruning, and storage and even selecting the best fruits. Theophrastus, the Greek philosopher had noticed and illustrated the differences between the domesticated sweet cultivated apple

from astringent wild types.

### 1.3 The historic era

The expansion of the Roman Empire helped in spreading the cultivation of the domesticated sweet apple within Europe. Researchers believed that hybridization with native crab apple *Malus sylvestris* occurred during that period (Luby, 2003). Many apple varieties were documented by the Roman naturalist and philosopher Gaius Plinius Caecilius Secundus, and by the first century had achieved a significant place in Roman cuisine, medicine and as well as ornament.

The Roman Goddess Pomona, who was the keeper of orchards and fruit trees, was also known as the Goddess of Apples. When the Roman Empire was overturned, the skills Romans had developed were preserved as part of the larger world of eastern and Celtic Christianity (Morgan and Richards, 2002).

### 1.4 Contemporary era

With the upsurge and extension of Christianity and Islam over the next several centuries, apple trees and its fruits received careful attention. It was expected that farms and orchards would be completely destroyed through wars and conflicts, but some trees were saved and maintained in the abbey gardens throughout Europe and the orchards of Iberia. A basic monastic skill was maintaining apple growing and many abbeys established a large orchard with many *Malus* x *domestica* cultivars. A similar situation existed in the Muslim world, in the eastern Mediterranean and Iberia where the Quranic teachings encouraged growing fruit trees and sustaining their cultivation. Thus, with time grafting skills, trees training and methods of pruning developed rapidly and more efficiently (Luby, 2003). During these periods there was a gradual diminishing of the cultivation of native crab apples which were replaced with domesticated apple in the

diet and cuisines in northern Europe.

### 1.5 Taxonomical classification and botany

Apple taxonomical nomenclature went through stages of evolution as findings were improved and resulted in significant evidence of apple hybridization that leads to the current identification. In 1803, Borkhausen first described *Malus* × *domestica* as originating from a hybrid derived from *Malus. sylvestris* Mill., *Malus dasyphyllus* Borkh. and *Malus praecox* Borkh. (Luby, 2003). However, during the twentieth century, *Malus sieversii* (Ledeb.) Roem. was identified as the main progenitor species of the modern apple (Juniper et al.1998; Roach 1988; Way et al. 199, Kumar et al., 2018). However, the taxonomy of the genus *Malus* is multifaceted, and may require a future revision when interpreting new information presented from the molecular genetics findings (Robinson et al., 2001).

Apple (*Malus* × *domestica* Borkh.) is a deciduous fruit tree species mainly grown in temperate regions around the world between latitudes 38°- 52° or it could be grown at higher elevations in the tropics on all continents except Antarctica (Pua and Davey, 2007). The genus *Malus*, belong to the *Maloideae* subfamily of the *Rosaceae* family and the order Rosales (Kumar et al., 2018). The *Rosacae* family, also include pear, quince, and medlar. As a temperate fruit tree, apple requires an annual period of chilling to break dormancy for the next growing season.

Apple is cross-pollinated, and most commercial cultivars are self- incompatible and their seed requires a period of cold stratification prior to germination. There are 25 documented species of *Malus* native to Europe, Central Asia, and Eastern Asia and three in North America (Way et al., 1991). *Malus* species easily intercross and since they are self-incompatible the seed of *Malus* are almost interspecific or inter-cultivar-hybrids. These species are known by their fruits which consist of two to five carpels enclosed in a fleshy covering. Most, if not all, wild apple species bear small and bitter fruits however many species and interspecific hybrids are used as ornamental plants.

The currently cultivated apple is a product of interspecific hybridization and the binomial *Malus* x *domestica* has been accepted as the proper scientific name (Korban and Skirvin, 1984). Fruits of many other species other than *M.* × *domestica* had been also consumed as fresh fruit but had another usage such as processed, medicinal purposes and some are used as rootstocks (Ferree and Warrington, 2003).

### 1.6 History of apple rootstocks

The use of rootstocks was reported by Theophrastus around 320 BC who discovered the specific characteristics of a slow-growing type of trees and easily rooted apple trees. This led to the adaptation of easily rooted dwarfing clones and the so-called Paradise rootstock, (the Persian word for garden) (Janick 2005). Since then, the selection of apple rootstocks has been based on a potential feature that enhances apple production. The below-ground part of the apple tree had been always disregarded in term of horticultural benefits or interaction with the scion cultivars until the practice of grafting had been mastered.

Along with rootstock selection, a necessity arose to combine and fuse two or

more different genetic individuals into one productive tree. This was known as the art of grafting by the selection of a scion for its horticultural qualities and adaptation to the above-ground environment and joining it with a rootstock which was adapted to the soil climate. This technique that had been practiced for several millennia and linked with domestication and propagation. However, the identification of rootstocks architectural and its effects on apple production is only about 100 years old (Rom and Carlson 1987; Tukey 1964; Webster 2003; Webster and Warrington 2003).

During the seventieth century, selecting superior fruit qualities and special phenotypic characteristics were basically practiced by either wealthy or religious personnel who could afford time and resources at that time. Good horticultural observations along with propagation techniques and grafting have led to the selection of unique dwarf and precocious apple rootstocks (Fazio et al., 2015). Subsequently, apple growers started observing and considering grafting had helped them in selecting specific rootstocks suitable for their growing requirements and conditions. Gradually they began to recognize that rootstocks have the capacity to influence their scion productivity and whole tree adaptability to various environmental conditions.

During the Eighteenth century, the first recorded apple hybridization began in Germany by Diel and in England by Thomas Knight (Cummins and Aldwinckle, 1983a). Influenced by their successful hybridization, thousands of apple cultivars were introduced by growers and nurseries who collected seeds from homegrown orchards and by chance seedling found at random locations. By that time, many apple clonal rootstocks had been identified and have led the apple industry in Europe to shift from seedling-rooted trees toward the new improved grafted stocks propagated asexually by

cuttings or layering and stooling of mother plants (Cummins and Aldwinckle, 1983b).

### 1.7 History of apple rootstock breeding programs.

In the early 20th century two major breeding programs devoted to apple rootstock improvement were started just after the First World War, John Innes at Merton and East Malling Research Station in England (Staniland, 1923).

The East Malling Research Station conducted the first documented apple rootstock evaluation and breeding program founded in 1917 (Cummins and Aldwinckle, 1983a). They started by collecting and sorting out the previously introduced apple rootstock cultivars. By 1920, they were able to identify, described and distributed several clonal series of rootstocks (Webster and Warrington, 2003).

The East Malling Research Station first released the 'M' series that was selected from a collection of European (German, Belgian and French) apple rootstock genotypes known as Doucin, Paradise apples and Jaune de Metz some of which have continued to be planted throughout the world till today. This breeding and evaluation program produced about 15,000 plants in 1921 and increased to 500,000 in 1936 (EMR, 2014). Some of their releases didn't meet growers and market expectations in terms of horticultural practices and lack of tolerances to biotic or abiotic stresses. Most of the clonal rootstocks from this program were from an unknown origin and few were identified and selected by Rivers in 1820 from an open pollinated seedling of English Nonsuch (Tukey, 1964). The pedigree the most widely planted rootstock in the world Malling 9 or of M.9 is unknown but it was selected from that initial group of apple rootstocks cataloged in the early 1900s and that was already recognized during the 1700s and 1800s. The Malling series of rootstocks is considered to be the fundamental

germplasm collection for all apple rootstock breeding programs and a major source for dwarfing and precocity characteristics (Fazio et al., 2015).

According to a report published by East Malling research in 2014, M.9 has an estimate of 95% market share of eating apple trees in the UK and Western Europe. It also stated that 9% of the market share of eating apples grown in the U.S. and South Africa are grown on M.9 rootstock (EMR, 2014).

The primary characteristics for rootstock selection were ease of propagation and the ability, as a grafted tress, to produce a dwarf tree and bear early edible fruits (Lauri et al., 2006). By the early mid-twentieth century, researchers at several breeding centers throughout the world were searching for adaptation characteristics that allowed rootstocks to tolerate various climatic conditions (Fazio et al., 2015). As a result of some of those efforts, a wooly aphid *(Eriosoma lanigerum* ) resistance rootstock was developed which to solve a problematic issue for apple growers in the Southern Hemisphere (Wertheim, 1998).

During the late twentieth century, global apple rootstock selection objectives were aimed at the maximization of apple productivity per area. A major shift to high-density plantation systems was introduced and practiced by many growers and ultimately increased the demand for rootstocks to sustain this new planting scheme. Consequently, several breeding programs started in major apple growing countries such as; Canada, Czechoslovakia, Germany, Japan, Poland, Russia, and the United States. In general, they sought to breed for resistance or tolerance against certain pathogens, pests, and for adaptability to localized climatic conditions. Breeders were developing multiple resistance characteristics by emphasizing potential infection or infestation complexes

that could occur in most common orchard's conditions (Lamb, 1969).

The Pacific Northwest Fruit Tester's Association (PNWFTA) reported in 2002 that there were 57 apple-breeding projects in 26 countries focusing on both apple scion cultivars but only a few on rootstocks (Ballard, 2002). The Cornell/USDA-Geneva breeding program continues to be one of the few active rootstock breeding programs in the world. It's breeding protocols are based on a methodical search for germplasm with features to overcome the weaknesses of the Malling germplasm and to to hybridize the new germplasm with existing dwarfing and precocious rootstock (Cummins and Aldwinckle, 1983b, Fazio, 2014; Fazio et al., 2015).

### 1.8 Global Apple production

Apple trees are the most widely grown fruit crop (King et al., 1991) with global production of 76,209 million metric tons in 2017/2018 according to the USDA Foreign Agricultural Service (Table 1). It is in the top 4 deciduous fruits grown in 94 countries (Asghar et al., 2012)

Table 1.Major apple producing countries worldwide 2017/2018 (in 1,000 metric tons)

| **Global leading apple producing countries** | **Production in 1,000 metric tons** |
|---|---|
| China | 44,500 |
| European Union | 10,021 |
| United States | 4,653 |
| Turkey | 2,750 |
| India | 2,300 |
| Iran | 1,573 |
| Chile | 1,360 |
| Russia | 1,277 |
| Ukraine | 1,100 |
| Brazil | 1,045 |
| Other | 5,630 |
| **Total** | **76,209** |

However, the global fresh apple production was predicted to decline by losing

5.6 million metric tons to 68.6 million due to weather-induced losses in major apple production region in China and the European Union (USDA 2018).

A report by published by the United States Department of Agriculture's (USDA), National Agricultural Statistics Service, found that the fresh apple production in the USA in 2018 was about 5.0 million metric tons with a value of 3.2 billion US dollars with 328,200 bearing acres of fresh apples.

Prime apple growing land is being displaced by urban and industrial developments. Breeding novel cultivars and rootstock that tolerate the adverse growing condition affected by climate changes while meeting the global market demands will help stabilize productivity demands and the need to plant in sub-optimal locations.

### 1.9 Honeycrisp™ apple Origin

Honeycrisp™ was developed from a cross at the University of Minnesota apple breeding program in 1960as part of developing high-quality fruit and winter hardy cultivars. Initially thought to be a cross of ‘Macoun’ and ‘Honeygold’ cultivars, however, genetic analysis has demonstrated pedigree disagreement and showed that one parent is to be ‘Keepsake’ (Cline and Gardner, 2005) while the other being another MN selection MN1627. A study created a multi-family based dense and high-quality integrated SNP and using the apple 8 K Illumina Infinium SNP array, they verified that ‘Keepsake’ was one parent of Honeycrisp™ and ‘Duchess of Oldenburg’ and ‘Golden Delicious’ were identified as ancestors of the other unknown parent (Howard et al., 2017).

Honeycrisp seedling was first planted in 1962 at the University of Minnesota horticultural research center, in east-central Minnesota. Twelve years later, in 1974 it

was noted for potential propagation and the evaluation started by labeling it as MN 1711 at several locations in Excelsior, Morris and Grand Rapids, Minnesota, and at Geneva, New York. It was then patented in 1988 and released in 1991 (Luby and Bedford, 1992).

**Honeycrisp™ tree's characteristics**

Honeycrisp apple has an upright, spreading tree growth habit, with low vigor, spur bearing fruit development and high precocity. Its vigor varies based on soil type, rootstock used, environmental conditions, and cultural management. Generally, Honeycrisp™ is categorized as a weak-growing scion. It starts blooming mid to late in the flowering season and appears to be pollinated by any diploid cultivar in the same season such as 'Cortland', 'Empire', 'Redfree', and 'Fuji'(Bedford, 2001). Honeycrisp™ has been characterized as winter hardy with good growth in northerly climates of the United States (Luby and Bedford, 1992) and it was reported tolerating temperatures as low as –34°C (Cline and Gardner, 2005).

**Honeycrisp™ Fruit's characteristics**

Although it has been more than 25 years since Honeycrisp™ was first released, it is still gaining remarkable popularity among apple growers and consumers. This is due to their exceptional balance of crispiness texture, juicy, balanced flavor, and aroma (Zhang et al., 2010). Those characteristics provided growers with premium prices in the northeast US and eastern Canada (DeLong et al., 2018). The US Apple Association predicted that Honeycrisp would be in fifth place for America's favorite apple by 2020 and is expected to be the third-most-grown cultivar (Bloomberg, 2019).

However, this cultivar requires significant horticultural and post-harvest management to maintain sustainable and profitable yield. Several reports described that

Honeycrisp™ produces a very heavy crop load resulting in small size and poor-quality fruits in heavy bloom seasons (Forshey, 1986) followed by little or no crop the following year (biennial bearing) (Crasswellerr et al., 2005; Robinson and Watkins, 2003). Eventually, this has led to laborious flowers and fruit thinning. Additionally, the fruit has a very thin skin thus it requires gentle handling to store well. It is also prone to bitter pit, a physiological disorder that has long been associated with low fruit Ca content (Schupp et al., 2005, 2001), localized Ca deficiency in fruit, (Cheng and Sazo, 2018; Schupp et al., 2005, 2001, 2001). Honeycrisp™ also experiences fruit coloring problems, appearance defects, and susceptibility to a leaf disorder referred to as zonal chlorosis caused by excessive loading of carbohydrates in the phloem (Cheng, L., T.L. Robinson. 2006. Along with bitter pit, the fruit is prone to scald, soft scald, and a tendency to ferment due to skin permeability problems (Rosenberger et al., 2001)

### 1.10 Apple whole genome

The publication of the first whole genome sequence (WGS) for cultivated apple (*Malus* x *domestica*) has facilitated many advances in the understanding of molecular mechanisms of apple (Velasco et al., 2010). The apple whole genomic sequence (WGS) has been very informative in clarifying some aspects of the physiology of apple trees and aided in genetic improvement of apple cultivars and rootstock via breeding (Peace et al., 2019). Although the apple WGS decoded about 81% of the genome since the average length of the assembly was 604 Mb compared to the estimated genome size of 742 Mb (Velasco et al., 2010). The previous N50 for this original WGS was only 16.7 kb. Authors described this WGS as a "high-quality draft" which implied that it is not fully complete which raises a question about the other un-coded parts of the genome

that might introduce even more enlightening knowledge about not only apple cultivars but probably rootstocks. This should provide significant potential research on rootstock genetics to correlate gene functions and traits of interest value (Peace et al., 2019).

### 1.11 The Cornell-Geneva Breeding Program

The Cornell- Geneva rootstock breeding program can be traced back to 1953 when Karl Brase began his experiment at Cornell University in Geneva, New York, by growing 158 open pollinated seedlings from the very dwarfing M.8 rootstock and pollen parents of M.1-M.16 from the Malling series with 'McIntosh' and 'Northern Spy' and ‘Empire’ scion cultivars. More than 90 seedlings were discarded due to an infestation of woolly apple aphids (*Eriosoma lanigerum*) or infections with powdery mildew *(Podosphaera leuctoricha* (Ell. and Ev.) Salmon, or due to poor rooting ability. Assessing the growth of the remaining seedling showed a range of tree size from smaller than M.8 to standard vigor. Only a few seedlings had higher productivity than M.9 with 'Empire' and 'McIntosh' cultivars. He noticed some rootstocks, as dwarf as M.9, were inducing little fruiting in the scion. Another issue with those seedling clones was the tendency to produce heavy suckers and susceptibility to fire blight (Cummins and Aldwinckle, 1983b).

In 1969, Dr. James Cummins and Dr. Herb Aldwinckle originated the Geneva apple rootstock breeding program. Their objectives were to develop rootstock genotypes with improved propagation and to improve tolerance to biotic stresses that suppress apple production specifically for eastern North America. At the time, fire blight (*Erwinia amylovora*), and crown rot (*Phytophthora* spp) were destructive diseases. The program was actively led by Dr. Cummins until his retirement in 1995. In 1998 the

Cornell rootstock breeding program was jointly re-launched with the Agricultural Research Service of the United States Department of Agriculture (USDA-ARS). The USDA breeder and the lead scientist was Dr. William Johnson from 1998-2000 and from 2003 has been Dr. Gennaro Fazio with the collaboration of Dr. Terence Robinson, Dr. Herb Aldwinckle and Dr. Awais Khan from Cornell University. The program seeks to develop new productive, disease resistant apple rootstocks using modern selection and evaluation techniques.

It has been 50 years since the first cross of the Cornell Geneva apple rootstock series (CG) in 1969. During that time many selections and screening methods have been developed to evaluate each growth stage from propagation in the nursery to adaptability and productivity in the orchard.

Advanced rootstocks selections have been tested in orchard trials at Cornell AgriTech in Geneva, New York, and on growers' farms in multiple locations in the United States and around the world.

The Cornell-Geneva breeding program had successfully identified many accessions of *Malus* species that show resistance to fire blight (*E. amylovora*), *Phytophthora* root rot, and wooly apple aphid *(E. lanigerum* ). Those accessions have served as a diverse gene pool for the apple rootstock breeding program (Aldwinckle et al., 1976; Aldwinckle and Lamb, 1978; Cummins and Aldwinckle, 1983a).

Actively, the program has been evaluating the field performance of apple rootstock nationally and internationally. It also focuses on assessing rootstock resistance to many biotic stresses caused by pest and diseases. Moreover, the program's research objectives have been extended to other scion traits influenced by rootstocks by

implementing genetic mapping and marker-assisted selection (MAS) (Fazio et al., 2015).

## 2 Apple Rootstocks.

The root system functions have been well studied in regard to water absorption and acquiring nutrients from the soil while providing anchorage to the tree. The tree's root system utilizes photosynthetically captured energy produced in the above-ground parts and transported to the root as stored sugar molecules. This energy is used to actively absorb nutrients and transport them to the above ground portion of the tree. This uptake of nutrients is done in some cases by interacting with rhizobium fungi in the soil (Blok et al., 2017).

The success of an apple orchard has been linked to proper rootstock selection which establishes a foundation for fruitful and sustainable production. Rootstocks are a key element in high-density orchards systems (Perry and Byler, 2001).

### Definitions

The apple rootstock is defined as the lower part of the apple tree which encompasses the root system and a small part of the lower trunk. Typically a modern apple tree is a product of combining two different genotypes by means of grafting a rootstock with a bud or stem piece of an apple variety. This technique that was practiced a long time ago when plant cultivation started (Autio, Hayden, Micke, & Brown, 1996). A third part, the inter-stem, is occasionally incorporated to be as an interface between the rootstock and the scion. Historically, rootstocks have been used mainly to aid in apple propagation in which is was impractical to propagate selected apple cultivars on their own root (Webster and Warrington, 2003).

## 2.1 Rootstock-to-scion interaction.

The effect of apple rootstocks and their influences on the scion cultivar has been known and available for growers for at least for two millennia. However, recogning the full potential of a rootstock's ability to control the scion growth and cropping happened about 100 years ago (Webster and Warrington, 2003). Although extensive studies have investigated the interaction between rootstock and the scion, there is little information about how rootstocks convey their effects to the scion (Jensen et al., 2012). The relative effect of different rootstocks on growth and fruiting of the scion remains similar for the majority of scion cultivars (Hirst and Ferree, 1995). However, the inherent vigor of the scion cultivar influences the final tree size on any particular rootstock (Webster and Warrington, 2003). Numerous studies have attempted to explain how apple rootstocks influence the growth and cropping of the grafted scion cultivar. Nevertheless, it is clear that rootstocks do control the vigor and precocity of the scion and its growth pattern leading to differences in production efficiency and differences in fruit quality. Moreover, rootstocks are being used to allow apple production in unfavorable growing conditions in relations to soil or the climate and tolerating pest and diseases. (Janick, 2015a). Although the mechanisms by which rootstocks influence the scion are not fully understood, what follows is a brief description of some effects of the rootstock on grafted apple cultivars.

**Controlling scion vigor and cropping**

Numerous studies have shown that apple rootstock control tree size, promote precocity, increase yield, maintain productivity for a long time, produce high-quality fruits and tolerate some of soil biotic and abiotic stresses (Crassweller et al., 1989).

Webster reported that trees grown on dwarfing rootstocks or interstocks produce earlier, more consistently and have higher crops (Webster, 1994). The complex traits of rootstocks are also affected by environmental conditions, scion genotype, and growth parameters (Foster et al., 2015).

Many theories have been proposed in attempts to explain the dwarfing effect on apple scions of some rootstocks (Lockhard and Schneider, 1981); however, recent genetic mapping work has identified two major genes are involved in the dwarfing effect of rootstocks. A genetic mapping study of populations from the Geneva breeding program confirmed the effect and location of Dw1, a dwarfing locus found on chromosome 5 of the apple genome found in M.9 rootstock (Pilcher et al., 2008). The inheritance of this one locus did not consistently explain rootstock vigor in breeding populations, and another locus responsible for apple tree vigor was identified as DW2 and found on chromosome 11 of the apple genome. The effects of these loci on tree vigor has been confirmed in various breeding populations using another scion cultivar (Fazio et al., 2014). Foster identified the majority of dwarf apple rootstock as dependents from the same genetic source (Foster et al., 2015).

Warner compared the invigorating rootstocks with dwarfing rootstocks/ interstocks and noted a reduction in the speed of shoot growth extension during the growing season and an earlier end of shoot extension during the summer or beginning of autumn. This influenced tree behavior and encouraged better horizontal branch angles. More horizontal angles are believed to be a result of the dwarfing rootstocks effects in reducing the trees' size of (Warner, 1991). Dwarfing rootstocks have the ability to enhance effective light interception and biomass partitioning for fruit production

instead of vegetative growth that significantly enhanced the apple productivity per unit area (Tustin & Hooijdonk, 2013).

**Influence of rootstock on hormones**

There are several studies on dwarf apple rootstocks that suggested rootstock could change the ratios and concentrations of the growth hormones, such as auxins, gibberellins or cytokinins, and possibly also lowering inhibitor hormones, such as abscisic acid, which are translocated within the tree (Adams et al., 2018). They found that the rates of basipetal auxin translocation was less in dwarfing than vigor rootstock stems. Furthermore, they found the ratio of abscisic acid to auxin was higher in the bark of dwarfing rootstocks and that differences in cytokinin translocation rates were significant (Kamboj et al., 1997; Soumelidou et al., 1994). Researchers studying the production and transportation of plant hormones within the tree found that rootstock influences the partitioning of hormones (Kamboj et al., 1997; Soumelidou et al., 1994). Studies from the molecular genetics of apple rootstocks show that rootstock has the ability to regulate the production of certain proteins in the scion cultivar (Fazio et al., 2014).

**Influence of rootstock on scion acclimation to environmental conditions**

Frequently, apples are grown in unfavorable conditions for optimum growth. The unavoidable climate conditions such as severe weather, very cold winter or dry summer directly impact apple growth. These conditions often cause the death of apple trees on many rootstocks and there is a huge demand for cold hardy rootstocks to sustain apple production. In the past 30 years, many apple growers experienced changes in climate with a shift to warmer summers, milder winters, and more intense rainfall. Climate

change may help growers in a colder area, but the unpredictable climate effects might be adverse (Ledford, 2013). Some apple production areas have reported a decline in their production due to climate change; India (Singh et al., 2016), Australia (Parkes, 2017), Italy (Eccel et al., 2009) and the USA (Ledford, 2013). A discussion of how apple rootstock influences scion cultivar in tolerating extremes in climate is below.

Cold

Over the past decade, most research on cold tolerance has emphasized the use of winter hardy rootstocks to support scion tolerance to cold winters by providing roots and trunks (shanks) of the rootstock with greater tolerance to very low temperatures (Mirabdulbaghi et al., 2010; Webster, 2003; Wildung et al., 1973). Several studies have assessed cold hardiness of apple rootstocks (Bite and Drudze, 2000; Cline et al., 2012; Moran et al., 2018; Privé et al., 2001). Wildung et al., (1973) compared field survival of several Malling clonal rootstocks (M.7, M.9, M.26, M.104, and M.106) under several cultural practices during cold winter conditions. They found that under clean cultivation with snow removal, M.26 was found to be the most hardy and M.7 the least hardy of the five rootstocks tested. (Wildung et al., 1973)

Zagaja compared the level of winter hardiness of apple rootstock from the Polish apple rootstock breeding program and found that P.2 and P.22 were considerably more winter-hardy than M.9 while they were equal to A.2 or Antonovka seedlings (Zagaja, 1981). A study by Moran et al., 2018 to identify genotypes of apple rootstock vulnerability to low temperature found varying degree of injury when measuring xylem, phloem and cambium browning. They reported that ‘M.7’, ‘M.9’, ‘G.935’, G.4011, G.4292, G.5087, and V.5, had partial xylem injury, whereas ‘M.7’, ‘G.41’, ‘G.214’, and

G.4011, had extensive xylem browning when subjecting to low temperatures (Moran et al., 2018). Evaluation of field performance of Geneva apple rootstocks reveled that G.41, G.11 and G.16 showed a good cold hardiness. (Robinson et al., 2006)

Drought

Sensitivity to drought is considered a major challenge to optimal apple production and may be mitigated by selecting the proper tolerant rootstocks (Higgs and Jones, 1991). Atkinson evaluated apple rootstocks in a drought experiment and found that rootstocks exhibit a large difference in shoot and root dry matter and root length. They also noted that dwarfing rootstocks tend to have smaller amounts of both coarse (>2 mm diameter) and fine roots (<2 mm diameter) than more vigorous rootstocks (Atkinson et al., 2003).

Rootstocks provide drought resistance to the scion cultivar by modeling scion stomatal closure, improved hydraulic conductance, recovery from embolisms in the xylem, and shift in assimilate partitioning to root growth (Tworkoski et al., 2016).

Dwarfing and very dwarfing rootstocks, such as Mark, P.22, and M.27 are more sensitive to drought while some semi-dwarfing rootstocks, such as J.9, showed some tolerance in drought conditions (Webster, 2003). Wang compared two apple rootstocks *Malus prunifolia* and *Malus hupehensis* in terms of tolerance to abiotic stress and focused in the leaf ultrastructure and responses by their antioxidant defense systems. They reported that a considerable ultrastructural alteration in organelles was observed when subjecting these rootstocks to a drought of 12 days. Their result showed that trees of *M. prunifolia* retained their cell structural integrity better than trees on *M. hupehensis* concluding that *M. hupehensis* was more vulnerable to drought than *M. prunifolia*

(Wang et al., 2012).

Soil temperature

Heide and Prestud (2005) found that consistent low soil?? temperature (< 12 °C) induced a cessation of growth and was related to dormancy induction in apple and pear. They found that rootstocks M.9 and A.2 ceased growing entirely at 9°C and 12°C, while MM.106 and B.9 continued growing slightly at 12°C for several weeks. Another study identified the optimal root's temperature for the development of the root system varies between rootstocks and found that 25°C is optimal for most rootstock clones, but 30°C was optimal for Malling series rootstocks (Gur et al., 1976). Lane investigated the effect of temperature on initiation stages of adventitious root and showed that temperatures below 28°C decreased IBA- and NAA-induced rooting in apple cultures (Lane, 1978).

Hydraulic conductivity.

Tworkoski and Fazio examined Geneva and Malling rootstocks and found that trees on G.935 and G.41 rootstocks had the most height and diameter growth and the maximum hydraulic conductivity. They concluded that trees grafted on G.11 and G.5087 may be better acclimatized to dry environments due to their size and higher ABA concentration.

This view was supported by Atkinson who found that the conductivity of the whole plant and its parts was increased by vigorous rootstock. While the scion grafted on vigorous rootstocks show less hydraulic resistance than trees grafted on dwarfing rootstock (Atkinson et al., 2003).

At the molecular level, a transcription factor of the dehydration-responsive element-binding protein (DREB1/CBF-type) is known for its role in tolerance to low

temperature, drought, and high salt stress. Yang et al. (2011) found and described *MbDREB,* a novel gene encoding a *DREB1* transcription factor from a dwarf apple, *Malus baccata.* The expression of *MbDREB1* was found to be prompted by cold, drought, and salt stress, as well as response to the exogenous abscisic acid ABA.

**Rootstock effect on nutrient uptake**

Apple root systems have low root length per volume of soil, but different rootstocks have different root architecture. Thus rootstocks react differently in response to soil chemical and structural properties by which the root system can influence water and nutrients uptake (Marini and Fazio, 2017).

Several studies have used field data to examine the effects of rootstock on apple tree nutrient level (Atkinson and Wilson 1979; Fallahi et al. 1984; Giorgi et al. 2005; Merwin and Stiles 1994; Weinbaum 1988; West and Young 1988), rootstocks response to soil's pH and soil type (Fazio et al., 2012a) and how at the molecular level rootstocks affects nutrient partitioning in the grafted scions (Fazio et al., 2013).

Past research has assessed the influenced of rootstock on the scion nutrient uptake by providing a uniform measurable media component for all rootstocks to grow in and assessing the absorption/transport to the above-ground portions of the tree by measuring nutrient concentration in leaf and fruit tissues (Fazio et al., 2018). However, few studies have focused on investigating the specific genetic role of apple rootstock on macro- and micronutrients absorption and translocation from the roots through the scion leaves and to the fruit (Fazio et al., 2015). Apple rootstocks have been evaluated to determine the optimal concentraton of nutrients when grown in varies range of orchard conditions to make fertilizer and soil amendment recommendations (Chun & Fallahi,

2002; Fallahi et al., 2011; Fan & Yang, 2011; Neilsen, Neilsen et al., 2008; Vaysse,et al., 2000).

Apple rootstocks differ in their inherent genetic ability to forage for nutrients and to translocate them to another part of the scion and ultimately influencing fruit quality at harvest and postharvest (Andziak and Tomala, 2004). The genetics of the rootstock contribute significantly to growth patterns and absorbance efficiencies of the root system (Wells and Eissenstat, 2001). In addition soil properties and soil pH actively interact with rootstock genetics to affect scion nutrient status (Fazio et al., 2012b).

Fazio analyzed quantitative traits of apple rootstocks from a breeding population and found a quantitative trait loci (QTLs) for leaf mineral concentrations of potassium (K), sodium (Na), phosphorus (P), calcium (Ca), zinc (Zn), magnesium (Mg), and molybdenum (Mo) (Fazio et al., 2019). Another study showed that variable gene combinations in the rootstocks causing changes in plant nutrient concentrationns would ultimately affect fruit size and quality (Hirst and Flowers, 2000). Hence productivity and disease resistance of apple trees are interconnected to rootstock nutrients uptake (Fazio et al., 2015).

Several studies have been conducted to analyze the differences within rootstocks in nutrients uptake efficacy (Cheng and Raba 2009; Fazio, Robinson, and Aldwinckle 2015; Neilsen and Hampson 2014). Apple rootstocks that are more efficient in absorbing and translocating calcium, may be capable of lessening the postharvest disorders like bitter pit (Val et al., 1998).

**Rootstock response to soil pH**

The ambiguity concerning the complex relationships between soil's chemical

properties and other soil quality factors hinders the determination of what is the optimum soil pH to maintain a healthy apple trees? The effect of extreme soil pH on apple tree growth and nutrients availability has been the subject of several studies. Raese (1992) pointed out that knowing the soil pH is an important factor when applying fertilizer to obtain a precision nutrition program of fruit trees. Fazio et al. (2021b) compared the effect of soil pH and type on the growth and nutrients absorption of 33 different experimental and commercial apple rootstocks. Their results validate that rootstocks showed a varying pattern of growth in response to soil pH. They found that the growth of CG.3007 and CG.5257 did not significantly differ by soil pH while the growth of CG.6589 was optimal at pH of 5.5 while the growth of G.41 and MM.111 was ideal at pH 7.5. Their study suggested that G.41 may be well adapted to even higher soil pH (Fazio et al., 2012b).

Cavallazzi used mycorrhizal fungi to mitigate the negative effect of acid soils on apple rootstock growth and found that soil pH significantly affected all growth parameters except branch dry weight (Cavallazzi et al., 2007). Hoyt and Neilsen (1985) explored the relationships between tree growth (trunk circumference), and soil pH and found growth was highly variable but correlated well with the soil's pH measurements.

**Adjusting scion flowering developments.**

Certain apple rootstock influences floral density, flower quality, flowering time and flowers sensitivity to frost (Durner and Goffreda, 1992).

Flowering precocity: most of the dwarfing rootstocks are known to induce precocious flowering of the scion cultivar. This could be related to their indirect effect on branching angles which correlates with horizontal branching which increases

flowering in apple (Robbie et al.,1993). Others have proposed that partitioning of assimilates and plant hormones in young trees influences the precocity of flower production (Webster, 1995). Dwarfing rootstocks also have the ability to induce changes in dry weight distribution to increase reproductive development and ultimately, flower and fruit production (Atkinson and Else, 2001). A recent study explained the influence of branch angle on flowering due to increased the total sugar concentration and the C/N ratio in the shoot terminals of the bent branches (Zhang et al., 2017). Thus, rootstocks producing open angle branches would also induce flowering precocity.

Flower density: Rootstocks enhance flower density by altering the scion growth to produce many short shoots with terminal flower buds. Another tactic, is increasing the proportion of floral axillary bud on the one-year-old wood relative to vegetative buds. Dwarf apple rootstocks can increase the number of floral clusters among spurs, or terminal and axillary buds on shoots as well as improve fruit set (Ferree, Hirst, Schmid, & Dotson, 1995) Some rootstocks like B.9, G41, G.202, G.214, and G.935 increase flowering density by increasing flowering spurs on second year or older wood or by increasing the number of flower buds per spurs and the number of flowers at each floral bud (Hirst and Flowers, 2000).

Flower quality: Rootstocks incluence flower quality by maintaining the availability and longevity of flower ovules and the synchrony of flower development. Rootstock influences and modify all factors that contribute to producing flowers and consequently their ability to set and retain fruits. This results in delaying the opening of the axillary flowers formed on the one-year-old wood. The axillary flower will result in shorter “effective pollination periods” (EPP) (Jackson and Hamer, 1980).

Flowering's time: Several studies reported that rootstocks influence the time of onset of flowering by manipulating the chilling hours or degree day of heat accumulation requirements for floral buds of the scion (Durner and Goffreda, 1992; Hirst and Flowers, 2000; Seleznyova et al., 2008). They found that M.7 apple rootstock required a lower chill unit accumulation and growing degree day heat accumulation when compared with M.26, M.9, or MM. 106. Their study was designed to explain these effects by measuring the differences in cytokinin supply to the scion buds (Webster, 1995). A recernt study on the chilling requirement and budburst found that rootstock influences budburst in response to varying chilling hours. They reported that G.213 induced more budbreak on 'Maxi Gala' than M.9. Other Geneva rootstocks also stimulated more budbreak of 'Maxi Gala' than M.9 when receiving only 800 chilling hours. Under 600 winter chill hours, G.814 and G213 achieved budbreak earlier than G.202 and M.9 for 'Maxi Gala'. (Macedo et al., 2018).

Frost sensitivity: Rootstock modify the flowering time by either advancing or delaying flowering. This may increase or decrease the risk of frost damage. Webster (1995) found that apple rootstocks may influence the tolerance of individual flowers to frost damage.

**Rootstock influence on scion fruit quality**

Several lines of evidence suggest that rootstocks can also influence apple fruit storage ability; however, this effect was difficult to conclusively prove due to many confounding factors (Autio et al., 1996). Rootstocks, like M.9, were reported to increase fruit size while others, such as M.27, have been reported to reduce it. Fazio used Honeycrisp cultivar to test the effect of rootstock on fruit nutrient concentrations. They

found that some rootstocks increased zinc concentration in leaves, and this led to reduced fruit calcium. They concluded that; CG.4002, CG.6976, CG.4814, G.16, G.214 and M.7 aided significantly in obtaining higher levels of Ca in both leaves and fruit of 'Honeycrisp'. However, tree on M.9 showed very low fruit calcium (Fazio et al., 2018). Another study compared the influence of apple rootstocks on the phenol content of the fruit and found a higher content of all phenolic compounds on super-dwarf rootstocks (P.61, P.22) while lower levels on dwarf rootstocks (M.9, P 62 and semi-dwarf M.26) (Kviklys et al., 2014).

**Rootstock influence on tolerance to biotic stresses**

The below-ground parts of the apple rootstocks are subject to many pathogen infections. Resistant or tolerant rootstocks are being used in areas with high pest and diseases pressure (Janick 2015a).

Severe infection by bacterial or fungal pathogen results in tree death. Fire blight is very destructive disease in many apple-producing regions of the world caused by the bacteria (*Erwinia amylovora)* (Ferree et al., 1983; Perry, 1992). This disease affects primarily the scion infecting both blossoms and shoots, but rootstock blight is also a big problem since it results in death of the tree. Most of the traditional rootstocks ave very susceptible to fire blight resulting in annual loses of millions of dollar. Several new rootstocks are resistant to fire blight (*E. amylovora*). All released Geneva series rootstocks are either resistant or very resistant to fire blight. Other rootstocks that exhibiting some resistance include: Bemali, M.7, M.4, M.2, MM.104, B.9, B.118 and B.490 (Russo et al., 2007a).

Resistant rootstocks also able to transmit some of this resistance to scions

cultivars (Jensen et al., 2012). They identified 690 transcripts whose expression levels at the steady-state were associated with susceptibility to fire blight (*E. amylovora*). Out of the 690 transcripts, 39 had expression levels in the scion that strongly correlated with fire blight (*E. amylovora*) resistance (Jensen et al., 2012).

The most common damaging fungal disease to apple rootstocks is collar or crown rot caused by *Phytophthora* sp. (Wilcox, 1993). Due to its significance to the apple industry, several studies evaluated rootstock sensitivity to (*Phytophthora* sp.). Fortunately, many rootstocks show good resistance (P.22, G.65, JM.1, JM.5, JM.8 M.9, Ottawa 3, P.2, P.16, G.16, B.9, Mark, J.9 G.11, G.30, G.210, M.116, M.7 and 'Marubakaido).

Several field evaluations found that apple rootstocks have different levels of apple replant disease (ARD) tolerance. Several researchers reported that many of the Geneva® series apple rootstocks, including G.935, showed better growth performance at replanting sites than other widely used rootstocks like B.9 (Fazio and Mazzola, 2004; Isutsa and Merwin, 2000; Mazzola et al., 2009).

A study compared the performance of various apple rootstock under soil fumigation treatments against apple replant disease (ARD) and concluded that the growth of M.7, M.26 and G.16 was suppressed in non-fumigated soil. However, they noted G.30 and G.210 rootstocks grew equally well in both positions (Rumberger et al., 2004).

## 3 Rootstock evaluation

Rootstock evaluating is an important procedure to ensure characteristic stability and adaptability to each scion cultivar and local climate and soil conditions. Since

rootstocks differentially promote nutrient translocation to leaves and fruit of the grafted scion, the level of rootstock efficiency in taking up nutrients must also be evaluated. This can be achieved by assessing factors contributing to the roots nutrients uptake and nutrients transport and partitioning to the other part of the tree (Webster and Warrington, 2003). The evaluation of rootstock adaptability to a specific variety has been especiallyimportant with the variety 'Honeycrisp' which has many fruits quality defects and physiological disorders as a result crop load or imbalance mineral nutrient in fruit (Neilsen and Hampson, 2014; Robinson et al., 2009; Serra et al., 2016). New improved rootstocks could support the nutritional weakness of the scion cultivar (Fazio et al., 2018b).

**3.1 Commercially available apple rootstocks.**

The fundamental success of apple production systems depends substantially on the proper selection of rootstock since it is a major factor influencing the viability and sustainability of productive orchards. However, the choice of the appropriate apple rootstock to establish a new orchard itself require specific knowledge of the capabilities and limitations of each rootstock. From better growth and yield performance, dwarf tress size, resistance to biotic and abiotic stress, increased precocity, efficient mineral nutrients absorbent, and better portioning and translocation on nutrients from root to the grafted variety, and adaptability to higher planting densities (Autio et al., 2008).

There are many series of apple rootstocks from a different breeding program that commercially available and used in the apple growing areas worldwide. The list includes; Budagovsky (Bud or B), Cornell/Geneva (CG or G), Malling (M) & Malling Merton (MM), Michigan Apple Rootstock Clones (MARK), East Malling/Ashton Long

(EMLA). Ottawa (O), Pillnitzer Supporter (Pi), Poland (P) and Vineland (V).

Following is a description of the most commonly used and those under investigation in this study;

**B.9**: Dwarf rootstock from the Budagovsky breeding program in Russia, a result of crossing M.8 × 'Red Standard' (Krasnij Standard). This rootstock has been evaluated and widely used in many apple-growing areas. B.9 is a little more dwarfing and marginally more productive than M.9. Like other rootstocks in this B series, their leaves are red (Auvil et al., 2011; Crassweller and Schupp, 2005). B.9 is very precocious and winter hardy. It produces few suckers and requires support. It thrives in well-drained soil and is very resistant to *Phytophthora* crown rot and has shown greater fireblight resistant than M.9 (Russo et al., 2007).

**G.11®**: Dwarf rootstock a product from a cross between M.26 × Robusta 5 that was released by the Cornell Geneva apple rootstock breeding program in 1997. Depending on soil fertility and irrigation, trees on G.11 are similar in size to M.9 T337 and M.26 and equally precocious but more productive with very high yield efficiency. G.11 is reported with medium resistant to fire blight (*E. amylovora*) and *Phytophthora* crown rot. It is moderately susceptible to woolly apple aphids. This rootstock requires support in the early years and develops few burr knots and root suckers. G.11 grows well in most soil conditions. (Robinson et al., 2003)

**G.41®**: Dwarf rootstock similar in size to M.9 NAKBT337. Released by the Cornell Geneva apple rootstock breeding program in 2003 from a cross of M.27 × Robusta 5 made in 1975. G.41 is a highly productive rootstock with very high yield efficiency. It very precocious and is winter hardy but also does well in a warmer climate.

It produces no suckers or burrknots and requires tree support. This rootstock is highly resistant to fire blight, *Phytophthora* Crown rot, Wooly Apple Aphid (*E. lanigerum*) and it appears to be tolerant to replant disease (ARD). A five-year study shows that G.41 produces trees parallel in size to M.9, but with higher yield efficiency than M.9 and produces few root suckers. It also has excellent fruit size and induces wide crotch angles (Crassweller and Schupp, 2005).

**G.202®**: A semi-dwarfing rootstock the result from a 1975 cross of M.27 × Robusta 5 that produces a tree similar to M.26. It was released in 2002 by the Cornell Geneva apple rootstock breeding program. It was characterized as precocious and productive rootstock that carries resistance to fire blight (*E. amylovora*), *Phytophthora* Crown rot, Woolly Apple Aphids (*E. lanigerum*) and tolerant to replant disease (ARD). This rootstock is considered to be an appropriate selection for weak growing cultivars like Honeycrisp™. A study evaluated 'Liberty' scion grafted on G.202 reported that trees were about 50 percent smaller than M.7 but with much greater production efficiency (Crasswellerr et al., 2005; Robinson et al., 2003).

**G.214®**: A dwarfing rootstock resulting from a cross of Robusta 5 × Ottawa 3. It is about 30-35% of the size of the seedling tree. The vigor of G.214 is similar to M.9,Pajam2, and M.9Nic29. However, it is more productive than those rootstocks with high yield efficiency and good cold hardiness. Trees on G.214 need support to withstand extra fruit weight. It is resistant to fire blight (*E. amylovora*), wooly apple aphid (*E. lanigerum*) and *Phytophthora* root rot. It is tolerant to replant disease (ARD). (Tworkoski et al., 2016).

**G.935®**: A semi-dwarfing rootstock resulting from a 1976 cross between Ottawa

3 **and** Robusta 5 and introduced by the Cornell Geneva apple rootstock breeding program in 2003. G.935 produces a tree similar to M.26 (between M.9Pajam2 and M.26) in virgin soil and has very high yield efficiency similar or better than M.9. G.935 is precocious with excellent fruits size. It is a mid-winter hardy rootstock but produces some suckering. Since it produces a very productive tree, it requires support and it is well adapted to most soils. It produces wider branch angles in the scion (Crassweller and Schupp, 2005). G.935 is highly resistant to fireblight (E. amylovora), Phytophthora Crown rot has good tolerance to replant disease (ARD) but is susceptible to wooly apple aphid (E. lanigerum). (Robinson et al., 2003).

**M.9**: The pedigree is unknown, but it was selected in England from a group of French genotypes called "Jaune de Metz" in the late 1800s. M.9 is the most common and widely used dwarfing rootstock in the world. It prefers a well-drained site and requires leader support. It is very susceptible to fire blight (*E. amylovora*), wooly apple aphids, tolerant to crown rot and can develop burr knots. Many clones of M.9 have been developed and sold by nurseries, including;

M.9EMLA which is a virus-free clone produced by the East Malling/Long Ashton research stations. This rootstock produces a tree approximately 25-30 % more vigorous than M.9.

M.9 NAKB 337, is a virus-free clone produced by in the Netherlands and has become the mostly widely used a clone of M.9. It produces a tree with 5-10 % less vigor than M.9EMLA.

Pajam 1 and Pajam 2, are virus-free clones of M.9 produced in France. Pajam2 produces a tree with 35 to 40 % more vigor than M.9 NAKB 337.

M.9 RN 29 or Nic29 is another virus-free clone clone from Belgium which produces a tree about 30 % larger than M.9 NAKB 337 (Crassweller and Schupp, 2005).

**M.26-EMLA.26**: A semi-dwarfing rootstock from a cross between M.9 × M.16 (Metziner Ideal) at East Malling Research Station in England. It is in the intermediate vigor rootstock between M.9 and M.7 and produces a tree about 40 to 45 % of a standard tree. It is a highly productive rootstock thus needs some support in the early years but reported to be self-supporting after establishment. This rootstock is very precocious with heavy fruit bearing and good adaptability for close plantings and double rows. It is winter hardy but produces a few suckers and sometimes the bud union can be brittle. It tolerates most well drained and sandy soils but is susceptible to *Phytophthora*. and highly susceptible to fire blight (Crassweller and Schupp, 2005).

**MM.106**: A cross of M.2 × Northern Spy by the John Innes Horticultural Institute and the East Malling Research Station in England. It is a semi-dwarf rootstock, slightly larger than M.7 which produces a freestanding tree. It is a precocious and productive rootstock. Trees on MM.106 are resistant to wooly apple aphid (*E. lanigerum*) but is susceptible to fire blight (*E. amylovora*), collar rot (*P. cactorum*) and is not recommended for poorly drained fields. A study shows that Delicious cultivars on MM.106 are susceptible to apple union necrosis caused by Tomato ringspot virus (ToRSV) (Crassweller and Schupp, 2005).

**O.3**: Is a semi-dwarfing rootstock that was bred by the Agriculture Canada Research Station in 1974 as a cold hardy rootstock. It is a cross of 'Robin' crab × M.9. Trees similar to the size of M.9EMLA but smaller than M.26. It is resistant to collar rot (*P. cactorum*) but susceptible to fire blight (*E. amylovora*), woolly apple aphids and very

susceptible to apple mosaic virus. O.3 is an older rootstock and is not currently propagated by apple rootstocks nurseries. (Crassweller and Schupp, 2005).

**P.18**: Is a semi-vigorous rootstock which resulted from a cross of M.4 × Common Antonovka and was released by the Research Institute of Poland. It is not a dwarf rootstock and produces larger tree about the size of MM.111. However, it is tolerance to fire blight (*E. amylovora*) and resistance to collar rot (*P. cactorum*) and perform well in wet or heavier soils.

**Supporter 4** (Pi.80), a semi-dwarf rootstock from Dresden -Pillnitz, Germany. It is a cross between M.9 × M.4 and is similar in size to M.26 with the same anchorage features. It has a better yielding capacity than M.26 and MM.106 but lower than M.9. It has good winter frost resistant and resistance to woolly apple aphid (*E. lanigerum*), crown gall (*Agrobacterium tumefaciens*) but is susceptible to fire blight (Fischer, 1997).

**V.1** Released by the Vineland station breeding program in Ontario, Canada. It is a product of open-pollinated hybrids of 'Kerr' crabapples and M.9. Tree size is similar or slightly bigger than M.26. However, its yield efficiency and fruit size are equal or larger than M.26. V.1 was reported to be highly resistant to fire blight (*E. amylovora*) with little production of suckers (Crassweller and Schupp, 2005).

**4 Molecular interaction between rootstock and scion cultivar.**

Apples are heterozygous thus seed propagation will not produce a true to type tree and apple cuttings are difficult to root. Thus, rootstocks have been used for more than 2000 years to propagate apple genotypes with desirable characteristics (Marini and Fazio, 2017). The past century many research projects helped understand the physiological changes in the scion that were induced by the rootstock. Recent molecular

techniques have been utilized to identify genes responsible for rootstock effects on the sicon. The availability of the whole genome sequence (WGS) for cultivated apple (Velasco et al., 2010) and the molecular genetic maps (Liebhard et al., 2003) along with the quantitative trait mapping (Fazio et al., 2014) have been used as a references in explaining the molecular interaction between rootstock and scion cultivar in apple. The following topics are just two examples of how molecular explanations are addressed in understanding the influence of apple rootstock on the scion cultivars.

Biotic stress

Several studies found that rootstocks stimulate the activation expression of genes in the scion responsible for tree architecture and disease resistance (Jensen et al., 2012, 2011, 2003). Another study used an apple DNA microarray to investigate the gene expression patterns in 'Gala' cultivar grafted on different rootstocks to examine the susceptibility to fire blight (*E. amylovora*). They identified more than 100 genes with expression levels correlated with fire blight susceptibility of the scion/rootstock combinations (Jensen et al., 2011).

Drought and Heat stress

The heat shock transcriptional factor (Hsf) gene family was found to differ in gene expression by the influence of the rootstock when comparing Gala scion on M.7 and M.9 rootstock (Jensen et al., 2003). They identified the double frequency of genes with homology to stress-related genes in Gala scion on M.7 compared with a single occurrence in Gala trees on M.9 rootstock. To determine the molecular interaction of apple rootstock, Shen (2001) identified clone from 'Gala' scion on M.7 with homology to HVA22, a stress-regulated gene (ABA, drought, salt, cold) family member, was

associated with stress tolerance. Another 'Gala' clone was reported to have homology to SP1/POP3, a protein implicated in drought tolerance and Hsp20, a heat-shock protein. By comparing leaves, blossoms, and fruit, Giorno et al. (2012) identified five *Malus domestica* heat shock families (MdHsfs) on Golden Delicious variety grafted on M.9 rootstock.

The apple genome reveals that it comprises of 25 full-length Hsf genes, which are significant regulators in sensing and signaling different environmental stresses (Velasco et al., 2010). Rootstock breeding programs could benefit from this information to facilitate the improvement of rootstock to increase heat stress tolerance (Marini and Fazio, 2017).

## 5 Effect and Interaction of soil pH on the rhizosphere.

### 5.1 Soil pH

Plant roots absorb water which often contains dissolved mineral elements. The availability of these nutrients is partially controlled by the soil pH (Figure 1). pH is a measurement of the acidity and alkalinity of a solution and calculated by the concentration of hydrogen ions ($H^+$). The pH scale ranges from 0 to 14, where an acidic solution has a pH of less than 7.0, and an alkaline solution with a pH more than 7.0. This scale is a negative logarithmic scale of the hydrogen ion concentration ($H^+$), represented as pH = - log ($H^+$) where each increment of pH has 10 times fold of hydrogen ions than the previous pH reading. When a solution's pH is measured as 4.0, it means it has a 10 times greater concentration of $H^+$ ions than a solution's pH 5.0 and has 100 times more hydrogen ions ($H^+$) than a solution of pH 6.0.

pH measurements characterize a balance of hydrogen and hydroxyl ions. A

solution with a higher concentration of hydrogen ions compared to hydroxyl ions has a lower pH while a solution with a higher concentration of hydroxyl ions compared to hydrogen ions has a higher pH (Pennisi and Thomas, 2005).

Soil pH is controlled by either acid or base cations as positively charged dissolved ions in the soil. The common acid-forming cations in the soil are hydrogen ($H^+$), aluminum ($Al^{3+}$), and iron ($Fe^{2+}$ or $Fe^{3+}$), while the common base-forming cations are calcium ($Ca^{2+}$), magnesium ($Mg^{2+}$), potassium ($K^+$) and sodium ($Na^+$).

### 5.2 Effect of Soil pH on nutrients availability.

At the optimum soil pH for growth essential mineral nutrients are generally available and plant root growth is good. At non-optimum soil pH, plant root growth is limited and their ability to explore greater soil volume is also limited and eventually stopped (Figure 1).

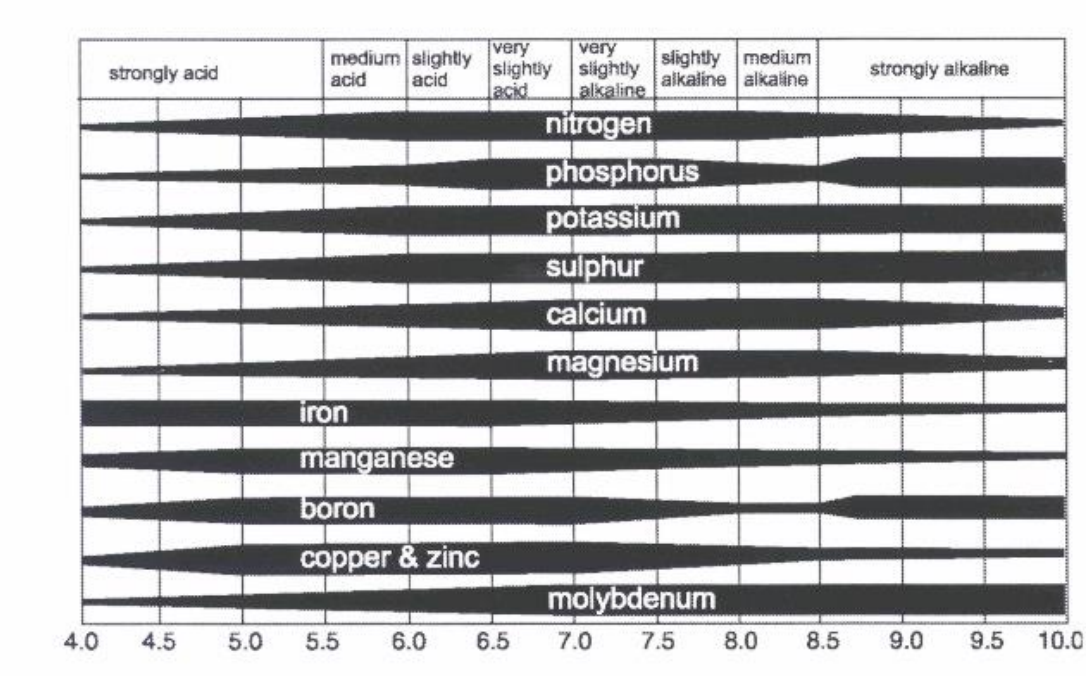


Figure 1.Nutrients availability in response to soil pH. (give citation of this figure)

The foremost limitations for plant growth on acid soils are toxic concentrations of mineral elements like Al and $H^+$. Furthermore, the acid condition causes less availability of other soil nutrients such as P and Mo and impaired uptake, of $Mg^2$ (Gillman, 1991). Al concentration inhibits root growth leading to more shallow root systems, which ultimately affect the ability for mineral nutrient acquisition and increase the risk of drought stress (Marschner, 1991).

In low pH soil, less than 5.5, there will is an increase in aluminum ion ($Al^{3+}$) concentration in the soil solution leading to reduced root elongation caused by altering the many functions of Ca including signal transduction pathways, plasma membrane and the cell wall integrity (Ma, 2007). Ultimately, this decreases the roots capacity to forage the soil for nutrients and water (Tang et al., 2002).

### 5.3 Optimum soil pH for the apple trees growth.

Soil pH affects nutrient availability by changing the form of the nutrient availability in the soil. It is widely accepted that plants growth is at optimum at pH range 5.5- 7.5 due to nutrient availability and mobility. Most authors agree to take this range as optimum for apple trees, however, this statement required rigorous evaluations and testing. It is possible that apple trees grow well at a lower pH than has previously been accepted. The effect of soil pH on apple tree growth was studied over 70 years back (Gardner et al., 1939). During that period, it was known that Pome and stone fruits grow well at pH range between 5.5 and 6.5 (Jonkers and Hoestra, 1978). In 1940 Edgerton studied the growth of apple seedlings by placing their roots in nutrients solutions and found that seedlings were tolerant to $H^+$-ion concentrations over a pH range from 3.6- to -6.6. Hoestra studied this effect using apple seedlings in pot experiments and found good growth of apple seedlings at pH 3.8 (Hoestra, 1968). Another study reported that apple trees had healthy growth at low pH levels range from 3.6-3.9 (Donoho et al., 1967). Another study examined native Chinese *Malus* species when grown at a range of pH values in hydroponic solutions. Three species from those population show the best growth at pH 5.5, while *M. sieversii* seedlings showed the best growth at pH 8.5. However, at pH 5.5, the growth of *M. sieversii* and *M. robusta* was negatively affected and stopped. Interestingly, the growth of *M. prunifolia* and *M. hupehensis* was inhibited at pH 8.5 (Fengchan Deng, 2012).

Lower soil pH increases the solubility of Al, Mn, and Fe leading to toxicity that slows or stops root growth (Fahr et al., 2013).

### 5.4 Effect of added nutrients to soil pH.

Amendment of soil properties either by raising or lowering soil pH is often used to create a more favorable growing condition. Adjustment of acid soils using lime to raise the soil pH to alleviate the negative effects of Al on root growth is essential to avoiding soil degradation and a drop in crop productivity (Conyers et al., 2003). The most common way to raise soil pH is by surface application of lime to the soil or by incorporation of lime into the soil not deeper than 10 cm. Lime has been also used to alleviate field nitrogen deficiency due to its ease of use and relatively low-cost. Surface application is believed to cause less damage to soil structural and minimum erosion risks. However, surface applications of lime to soils is slow slow and ineffective in amending subsurface acidity. Thus, it is highly recommended to use cultivars that can tolerate acidic condition to sustain production of those soils (Conyers et al., 2003; Scott et al., 2001).

Researchers found that drip-fertigating in orchards using ammonium nitrate ($NH_4NO_3$) led to faster soil acidification in the wet soil directly under emitters (Parchomchuk et al., 1993; Wójcik, 2018). They compared different soils with low pH buffering capacities (pHBC). Their result showed a strong acidification process happened under drippers which was associated with internal bark necrosis (IBN) disorder and consequently lead to a reduction in tree vigor and fruit yield (Ferree and Thompson, 1970; Hoyt, 1988; Wójcik, 2018). These symptoms are often related to the excess level of manganese (Mn) and aluminum (Al) in soil solution. Wójcik, (2000). found ammonium type fertilizers decreased the bulk soil pH and raised Mn leaf's level to a toxic concentration for apple, whereas $Ca(NO^3)^2$ increased leaf Ca level. Results

from their experiment concluded that the addition of ammonium-type fertilizers may accelerate soil acidification process at soil and rhizosphere level (Tagliavini et al., 1997).

### 5.5 Effect of soil's pH on Micronutrients:

Micronutrients or trace elements are affected by changes in the soil pH. When the soil pH is strongly acidic, some micronutrients become more mobile and can be absorbed in excess amount, causing potential plant toxicity (Clarkson, 1996). While when the pH is too high, the concentration of $H^+$ is low and micronutrients become less mobile and less available for plants to absorb, which results in deficiencies that limit plant growth (Haynes and Swift, 1986). This can be summarized as follows: the macronutrients (N, K, Ca, Mg, and S) are more available within a pH range of 6.5 to 8.0 except for P which is more available between pH ranges 6.0-7.0. Micronutrients (B, Cu, Fe, Mn, Ni, and Zn) become more available within a pH range of 5.0 to7.0 (McCauley et al., 2009) wheras micronutrient deficiencies symptoms of B, Cu, Fe, Mn, and Zn appear in many plant species at high soil pH due to insolubility which makes these nutrients unavailable to the plant (Mengel and Kirkby, 2001).

At soil pH values $\geq$ 8.0 micronutrient availability declines significantly as a result of cations such as Cu, Fe, Mn, Ni, and Zn become very tightly bound to the soil. At low soil pH there can be micor-nutrient toxicities for plants since base cations like Ca, K, and Mg are less bound to the soil and are likely to be leached (McCauley et al., 2009).

Porter and others found a decrease in phosphorus availability in alkaline soils (Porter et al., 1987). Other high soil pH effects can be observed with the altering of the

structure and availability of soil endo-mycorrhizal fungi which help root systems take up nutrients. In addition, in alkaline conditions, the solubility of Al and Pb is reduced becoming less thus reducing toxicity to the roots (Cavallazzi et al., 2007).

Raese (1992) reported a reduction of apple growth in the soils with lower pH or high salt concentrations. They found that apple trees develop bark measles with stunted growth and reduced fruiting when grown in very acid soils due to manganese toxicity. Stunted growth, iron chlorosis, and phosphorus deficiency were found on trees grown in alkaline soils. Apple cultivars which are sensitive to calcium disorders, show effects on fruit calcium levels due to soil pH (Marsh et al., 1996).

### 5.6 Effect of soil's pH on Electrical Conductivity (EC).

Nutrient availability is directly affected by soil pH in term of cation and anion exchange capacity (CEC & AEC) (Jones & Jacobsen 2005). Exchange capacity (EC) can be described as the soil's capacity to retain and supply nutrients to plant. When a field's soil has a negative net charge, the soil's cation exchange capacity (CEC) is greater than the anion exchange capacity (AEC).

Soil with high CECs will bind $Ca^{2+}$ or $K^{+}$ cations to the exchange sites of clay and organic matter particle surfaces. High CEC soil tends to have higher pH buffering capacity (pHBC) which increases the soil's capability to prevent pH fluctuations. Soil that contain a greater fraction of clay and organic matter usually have a higher CEC and buffering capacities than more sandy or silty soils. Since $H^{+}$ is a cation, it competes with other cations for exchange sites. When the soil pH is > 7.0, the concentration of $H^{+}$ is low and more base cations will adhere to the particle exchange sites and eventually will be less prone to leaching. However, when the soil pH is < 7.0, there is a higher

concentration of $H^+$ and extra $H^+$ ions will be available to be exchanged with the base cations thus, replacing them from the exchange sites and releasing them to the soil water solution. Consequently, exchanged nutrients are either absorbed by the plant or lost through leaching or erosion (McCauley et al., 2009).

### 5.7 Effect of soil's pH on Microbial activity.

Microbial activity and soil microorganisms are also affected by soil pH level. The soil activity of microorganism flourishes near-neutral pH conditions, however, each microorganism species has its optimum pH ranges (Aciego Pietri and Brookes, 2008).

Very acid soils with pH < 5.0 show lower microbial activity than neutral soils. Several studies reported that certain nitrogen-fixing bacteria and nitrifying bacteria which convert ammonium to nitrate are negatively affected by soil pH < 6.0 (Haby, 1993; Sylvia et al., 1998).

The effect of root-mediated pH changes is another significant factor in soil ecology sicne soil pH influences the physiology of the roots, rhizosphere microorganisms, and the bioavailability of soil nutrients. Roots respiration can produce carbonic acid in the rhizosphere which leads to localized reductions soil pH. Eventually, rhizosphere pH can be changed by the plant's root system and the accompanying microorganisms by redox-coupled reactions (Zhang et al., 2018). Many environmental constraints influence the dynamic progression of root-mediated pH changes in the rhizosphere (Hinsinger et al., 2003). Up to date studies are limited in explaining the contribution of root exudation and respiration in decreasing rhizosphere pH as a result of a build-up of the $CO^2$ concentration (Hinsinger et al., 2003)

# 6 Effect of Soil pH on Root Architecture

## 6.1 Definitions

The term “architecture” in a biological context represents the spatial configuration of a complex assembly of subunits which has some functional significance. A study elucidated that several contexts used the term “root architecture” to refer to diverse features of the root systems organization (Morris et al., 2017). The following definitions will explain the root architecture and distribution as described by Lynch (Lynch, 1995).

Root morphology refers to the surface structures of a root which consists of; the root hairs, root diameter, root cap and the folds of the root axis. However, the anatomical features of the root, such as cell and tissue organization are not considered an architectural part of the root.

Root topology refers to how the root branching is formed and the connection between root axes.

Distribution refers to the presences of the root in a positional gradient. Usually, studies of root distribution are focused on root biomass or root length.

Architecture refers to the spatial configuration of the entire root system (Figure2).

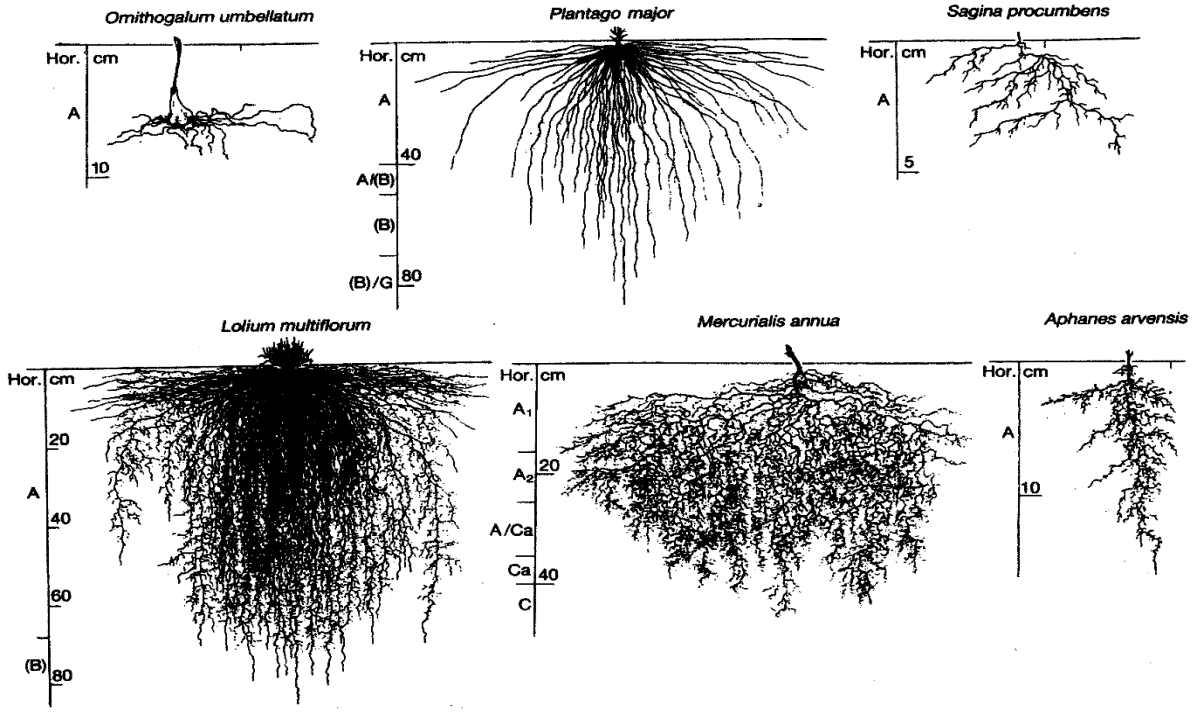


Figure 2.Example of different constructed root systems. Top row, little branched root systems. Bottom row, more freely branched on the (Doussan et al., 2003).

Most studies on root architecture have been focusing on the entire root system or a large subset of the root system of an individual plant and rarely include fine structural details such as root hair activity (Lynch, 1995). Considering multiple root axes, root architecture has been more significant than topology or distribution. Knowing the root architecture leads to understanding both topology and distribution. However, neither topology nor distribution can lead to predicting the other two root descriptors. Thus, measuring the root topology and distribution are more practical than root architecture and has been used more frequently in studying root formation (Lynch, 1995). The interactions between plant and soil certainly influence root growth in plants. Faget et al. (2013) showed that root growth is affected by root-root interactions leading

to a specific architectural pattern and root characteristics such as lateral root formation. Similar interaction can be also observed between the same species growing together.

### 6.2 Plant response to soil pH changes.

For many years, plant's response to varying soil pH was studied before understanding that rhizosphere pH can be altered by the roots by releasing $H^+$ or $OH^-$ to compensate for an unbalanced cation-anion uptake at the soil-root interface (Riley and Barber, 1971, 1969). Since then several published studies reported the effect of rhizosphere pH on plant's root systems and the role of soil pH on nutrient availability (Dakora and Phillips, 2002; Hinsinger et al., 2003; Y. Liu et al., 2004; Nye, 1981; Sarkar and Wynjones, 1982). However, the roots of some plants have the ability to accommodate and mediate the changes in soil pH (Dakora and Phillips, 2002). Many findings indicate that soil's pH has a critical role that influencing the availability of many nutrients, toxic elements, the physiology of the roots and rhizosphere microorganisms (Baligar et al., 2001; John et al., 2007; Qadir and Schubert, 2002; Zeng et al., 2011).

The process by which roots induce pH changes in the rhizosphere is by releasing $H^+$ or $OH^-$ ions to alleviate any unstable cation-anion uptake at the soil-root interface (Hinsinger et al., 2003). Foy reported that the additional $H^+$ competes with other cations for root absorption sites by interfering with ion transport and uptake, which ultimately causes leaking in the root membranes (Foy, 1992). While Poschenrieder found alterations of nutrient uptake play an important role in $H^+$ ion toxicity (Poschenrieder et al., 1995). A study found that the operation of an energized ATPase pump at the root plasma membrane was believed to be associated with maize roots adaptation to low pH

soil (Yan et al., 1998).

Kidd and Proctor, (2001) investigated the reasons for inhibition of plant growth at low pH and found that some plants are distinctly adapted to $H^+$ or $Al^{3+}$. They found that the reduction in plant growth at low soil pH was not related to a P deficiency. However, they found a maximum decrease of 24% between solution changing P concentrations when nutrients solution was analyzed.

Plants species vary in their responsiveness to nutrient deficiencies induced by alkaline soil pH. Moog and Brüggemann, (1994) identified a specialized enzyme that reduces Fe in some tolerant species that increase Fe uptake. An evaluation experiment of olive tree cultivars and rootstocks showed that the rootstock rather than the grafted scion contributed tolerance to calcareous soils (Alcántara et al., 2003).

### 6.3 Effect of soil's pH on bitter pit disorder.

Bitter pit is a physiological disorder related to an imbalance in minerals and uptake, so it is not surprising that it is also related to soil pH. Several studies has shown the fruit's calcium level is associated with apple fruit bitter pit (Cheng and Sazo, 2018; Donahue, 2017; Robinson and Watkins, 2003; Rosenberger et al., 2001; Schupp et al., 2005; Terblanche et al., 1980). Since the level of available $Ca2^+$ions in the soil strongly correlates with increased soil pH, a could be relation between soil pH and bitter pit of apple. It was found that high amounts of nitrogen increase the incidence of bitter pit, especially on soils with low pH (Jonkers and Hoestra, 1978; Nava and Dechen, 2009).

Honeycrisp™ is considered to be a major apple variety grown in the northern apple production area in the US and southern Canadian provinces. However, more than 50 % of young plantings have been developing bitter pit before or during storage (Cheng

and Sazo, 2018; Rosenberger et al., 2001). Imbalance of Ca with K, Mg, and P are also associated with bitter pit incidence (Cheng, 2016).

Several approaches have been used to control biter pit such as liming soils to obtain the optimum pH for apple growth. Another practice has been implementing calcium spraying, however spraying efficacy is highly variable between orchard blocks and within growing seasons (Cheng and Sazo, 2018).

Most recommendations to control bitter pit suggest an integrated management approach. This includes pre-planting strategies such as adjusting soil pH and proper rootstock selection to control tree vigor to maintain proper Ca partitioning. As well as strategies during the growing seasons including controlling crop load, maintaining adequate Ca, B and Z, maintaining stable irrigation and firmly controlling K, N, Mg, and P to balance Ca to K ratio (Cheng, 2016).

### 6.4 Effect of soil compaction on soil pH.

In compacted wet soils conditions, water will fill the few pore spaces left while removing oxygen. Thus, compaction affects the movement of water and air across the soil surface boundary. Infiltration of water is critical for plant and soil health. The lack of air will consequently cause changes in soil chemistry, which ultimately lead to nutrient unavailability or poor uptake. For instance, denitrification which is a bacterial process that converts soil nitrate into gaseous nitrogen which it then lost to the atmosphere occurrs often in compacted wet soils. In that condition, the soil will show a decrease in pH by which an acid condition is created, and nutrients become less available. (Beegle, 2006).

Głąb and Gondek (2014) found that compaction from a tractor changed some

chemical properties of soil and resulted in increased pH and EC on a Lucern field. Results published by Bhandral analyzed measurement period over the uncompacted soil and found a significantly higher pH. They attributed this effect to a low level of nitrification in compacted soils, resulting in the release of only a small amount of protons to the soil (Głąb and Gondek, 2014).

The effect of increased water content and decreased macro-porosity is reduced gas diffusion which lead to root aeration stress. Complex diversity in plant species in tolerating soil compaction could be considered as a response of the rhizosphere environment (Siegel-Issem et al., 2005). Another report found that higher soil strength due to higher bulk density was related to soil moisture content rather than to lime incorporation. Their result came from comparing wheatgrass grown in high-strength, acid soils or in acid soils where macropores help avoid acidity and high bulk density (Haling et al., 2010).

Another effect of soil compaction can be seen in conservation tillage systems when soil pH often is stratified because of the surface application of limestone and acid-forming nitrogen fertilizers and manures. This stratification can further influence rooting patterns, the availability of nutrients, and the effectiveness of herbicides (Beegle, 2006).

An experiment of soil compaction found that soil pH increased significantly with increasing compaction which resulted in less aggregation of the soil by increasing the concentration of coagulation and as a result in a collapse of minerals and the release of cations (Saiedyfar and Asgari, 2014). A similar conclusion by an earlier study showed that an increase in soil pH caused degradation of aggregates, clay particles to swell and

disperse causing the formation of crusting and reducing porosity and permeability. Another result was that increased soil pH increased nitrification and root exudation (Franzluebbers and Hons, 1996).

### 6.5 Soil salinity

Soil salinization has been defined as the accumulation of water-soluble salts in the soil to a level that affects the plant growth and reduces environmental health, and creates economics issue (Rapparini and Peñuelas, 2014). The effect of salinity starts early by disturbing the soil organisms' metabolism and thus reduces soil productivity. In later stages, it inhibits the growth of all vegetation and organisms living in the soil, eventually leading to converting fruitful and productive land into infertile and desertified lands (Fouda et al., 2018).

Many researchers have focused on soil salinity as important abiotic stress limiting apple production (Andrade et al., 2018; Pokharel and Zimmerman, 2016; Saleem et al., 2018; Taha et al., 2017). It has been reported that a high concentration of salt in the soil solution is linked to reducing leaf stomatal conductance, low chlorophyll concentration, and reduced leaf water potential and relative water content (Marini and Fazio, 2017). These series of alterations lead to suppressed leaf expansion and reductions in plant growth along with the accumulation of proline and soluble sugars in the leaf (Alizadeh and Alizade, 2013).

### 6.6 Effect of Soil pH on the acidification process.

The soil acidification is a natural process resulting from the combination of metabolic processes occurring in the soil such as respiration and water balance in the particular region (Coughlan et al., 2000). Soil acidity also occurs in areas with higher

annual rainfall and their incidents differ based on the landscape geology, soil texture, clay mineralogy and buffering capacity.

Soil respiration, a process of $CO_2$ production, made by animals, plant roots and microorganisms and other metabolic reaction combines with water and making carbonic acid. Although it is a weak acid, it is continuously produced in the soil. This acid dissociates leading to producing of ample $H^+$ ions, which replaces basic cations in sorption complex (Metternicht and Zinck, 2003).

Acidification or alkalization of soils occurs in all soils and happens through the buildup of hydrogen protons ($H^+$) transfer processes involving vegetation, soil solution, and soil minerals. This process could happen naturally and in a very slow manner as the soil gets weathered. However, the acidification process could be accelerated in heavy or commercial productive agriculture system (Breemen et al., 1983).

Several studies have been focusing on soil acidity and strongly alkaline soils, considering its significant importance due to the complications related to plant growth performance at extreme soil pH (Paul et al., 2018; Pokharel and Zimmerman, 2016; Tkaczyk et al., 2018). Acid soils are found in nearly 30 % of the world's total land area, and over 50 % of the world arable lands has been estimated as potentially acidic soil (von Uexküll and Mutert, 1995).

Acidity itself is not accountable for limiting plant growth. However, factors associated with soil acidity are considered to be a major plant growth limiting in some soil types (Kidd and Proctor, 2001). Such factors including the toxicity of $Al^{3+}$, $Mn^{2+}$ and low supply of N. Deficiency of P and Mo along with toxicity of phenolic acids are all associated with soil acidity. The Hydrogen ions by itself considered as causal for

poor growth. Normally bacterial populations favor a marginally acid environment. However, in highly acidic soil most of the beneficial soil bacteria population and survival can be hinder. As the soil gets more acidic, the auspicious for bacteria, earthworms, and many other soil organisms is ruined (Hollier and Reed, 2005).

Changes in soil pH impact the soil nutrients availability and their interaction. In low pH soil, many elements become unavailable to plants, whereas others such as iron (Fe), aluminum (Al) and manganese (Mn) become toxic to plants. On the other hand, in high pH soil, calcium bonds up phosphorus, making it unavailable to plants. Also, molybdenum (Mo) and boron (B) become toxic in some soils. (Havlin et al., 2016)

**7 Root-to-soil interaction.**

The interaction between plant roots and soil is a series of complex physical, chemical and biological processes. The chemical and physical background of the rhizosphere is the basis of the interaction. The processes vary from the small simple reaction of element fluxing in or out the root system to the complex biochemical reaction between soil microorganisms and root surface with high recognition specificity (Tinker and Barraclough, 1988). The rhizosphere itself is a dynamic environment with fluctuations in its composition due to root development and other degradation and mineralization of nutrients.

A vital characteristic of plant interactions with the environment happens below ground. However, due to limited available techniques to study the interaction below ground, the information on the interaction between root to root is less available than for above-ground interactions. Several studies have focused on the root interaction by tracking the above ground effects during an experiment. However, the results of the

below ground interactions were only measured at the end of an experiment using destructive methods.

Studying the underground dynamics of root architecture and growth needs a modern and non-destructive technique to facilitate measuring rhizosphere interactions over time. Root to root interactions can be analyzed by collecting repeated measurements and reading of root systems through their growth development by implementing non-destructive methods. (Faget et al., 2013).

### 7.1 Dynamic of Apple Root Architecture and Morphology

Several studies have shown that plant root systems can adapt dynamically based on nutrient availability and distribution by altering the three-dimensional deployment of their roots system architecture accordingly to soil types and conditions (Linkohr et al., 2002; López-Bucio et al., 2002). However, apple root architecture has received little attention on this topic.

The spatial distribution of roots and root morphology have a direct impact on root architecture and concentration of nutrients in the soil solution. Studies focusing on root morphology found abundant genetic variation in the root architecture of apple rootstocks (Fazio et al., 2015). This characteristic is believed to play an important role in controlling the overall tree size and productivity of the scion cultivar. Root distribution and architecture contribute to water and nutrients uptake and thus modulate root to shoot ratio (Fazio et al., 2015).

One valuable feature of the root system is its ability to produce high-density fine roots leading to increased root surface area and greater soil exploration and nutrient uptake. This fine root characteristic was found in many apple rootstocks and was

reported in several elite Geneva apple rootstocks (Fazio et al., 2015).

A study investigating the effect of phosphate uptake on apple root architecture found timely dynamic changes in root architecture and the capacity of phosphate uptake in response to varying phosphate solution (Weiguo Fan and Hongqiang Yang, 2008).

### 7.2 Root development

Plant roots undergo several development stages starting with the root tip zone, and continuing with the extension zone, root hair zone, loss of cortical cells and secondary growth (Tinker and Barraclough, 1988). Not all plant species follow the same sequences or stages with variation between monocotyledonous and dicotyledonous species. The same applies for the duration of the root stage activities that are more diverse among species as well as in different rhizosphere conditions. Generally, more root masses can be found in the topsoil and there is an exponential distribution of root density with soil depth (Tinker and Barraclough, 1988). Other factors may contribute to root distribution such as poor drainage and root metabolism that influences root development. Waterlogged soils create anaerobic conditions resulting in depleted oxygen available for soil microorganisms (Gerwitz and Page, 1974). Under anaerobic conditions, root metabolism is altered and contributes to the production of toxins such as $Fe^{++}$ or $H_2S$ that affect the root development. Typically, not all roots are affected at the same level. Some have the ability to use their roots as channels to supply oxygen to the rhizosphere (Armstrong, 1978). Root hairs are considered as extensions of the epidermal cell with their microscopic length varying from 0.1-5m and diameters from 0.005-0.025mm and amazingly they modify the root surface geometry. Despite their short life span of few days, a vast variation can be noted

on their length and density which depend on species. Their characterization also depends on the soil mineral compositions. Many studies show that lower concentrations of nitrate and phosphate in the soil lead to longer and denser root hairs when compared to higher concentration (Bhandral et al., 2007; Bloom et al., 1992; Neale et al., 1997).

### 7.3 Root-induced pH patterns

Root exudates are capable of modifying the immediate vicinity soil pH that ultimately affects the uptakes and availability of nutrients and phytotoxic metals (Kim and Silk, 1999). Several studies found that roots are capable of acidifying an alkaline medium in calcareous soil condition (Gérard et al., 2017; Higuchi et al., 2017; Hsieh and Waters, 2016). They can also increase the pH of an acidic soil medium (Läuchli and Grattan, 2017). Root induced soil acidification of the rhizosphere makes soluble macronutrients and the micronutrients more mobile.Thus, knowing the root-induced pH changes in the rhizosphere is a critical aspect when determining the rate of mineral uptake in plants and subsequently the concentrations in the fruit and seeds. (Kim and Silk, 1999).

A theory was developed by Nye to predict the plant-induced changes in field pH in the rhizosphere assuming that hydrogen ions will diffuse according to a concentration gradient from high concentration to low (Nye, 1981). He used an analytic approach to design a diffusion equation with flux over the surface of a cylinder to model rhizosphere pH as a function of distance, r (from the root surface); and time, t:

$$\mathrm{pH} = \mathrm{pH}\infty \left(\frac{aF}{2b_{\mathrm{HS}}D_{\mathrm{HS}}}\right) \ln\left(\frac{2.25D_{\mathrm{Hs}}t}{r^2}\right)$$

where (F) is the flux of $H^+$ (from the root surface); (a) is the root radius; (pH∞) is the initial soil pH;( $b_{\mathrm{HS}}$) is soil buffering capacity; and ($D_{\mathrm{HS}}$) is the soil acidity diffusion

coefficient.

Generally, soil water content and diffusion impedance account for variations in the acidity diffusion coefficient. From this fact, Nye's model assumes bigger effects on the pH from initial soil pH and from soil water content and a logarithmic dependence on time. Steep pH gradients were predicted within the rhizosphere profile, extending from 0·5–3·0 mm away from the root surface. This is expected when the acidity diffusion coefficient is high in a low soil buffering capacity. This model predicts the effect of mass flow in developing of the pH patterns and the presence of root hairs is neglected (Kim and Silk, 1999). They explained that combing the flux of surplus cations with the flux from the iron oxidation process sums up the total $H^+$. Observed values for soil properties were used with the observed estimates for $H^+$ flux and Equation 1 to predict spatially and temporal patterns of pH (Kim and Silk, 1999). This model has helped clarify how hydrogen ion fluxes associated with root metabolism would affect soil properties. Though it is still one-dimensional and predicts constant, spatially uniform $H^+$ flux (Kim and Silk, 1999).

## 8 Soil pH adjustment

### 8.1 Raising soil pH

The regular application of lime will increase soil pH by lowering the soil's acidity. Several options are available such as; adding calcium (Ca), or magnesium (Mg), to reduce the solubility of Al and Mn below the toxic level. Liming materials vary in their effectiveness. Calcium or magnesium carbonate are traditionally used and react with soil acidity to neutralize it. Liming materials have very slow movement into the soil without proper mixing. Field practices such as tillage increases the effectiveness of all lime materials by incorporating them into the rooting zone (Anderson et al., 2013).

Soil acidification is neutralized by the addition of hydroxides, carbonates, and silicates of Ca and/or Mg. The base anions in liming materials react with soil acidity $H^+$ to neutralize it. The most commonly used liming material that provides carbonate as the base is calcium carbonate. Calcium itself does not raise soil pH. For example, calcium sulfate (gypsum) and other additives contain Ca but do not contain a basic anion (carbonate, hydroxide, oxide, or silicate). Thus, they do not neutralize soil acidity (Spies and Harms, 1988).

### 8.2 Lowering soil pH

Reducing soil pH or soil acidification is a natural process that is enhanced by some field cultural practices, mainly application of sulfur, nitrogen (N) fertilizers in form of urea or ammonium sulfate or other soil amendments that contain ammonium-N (Adams, 1984). As soil acidification occurs, several chemical and biological properties of the soil also change. An important chemical change occurs in the acid soil, is the increase of aluminum (Al) and manganese Mn solubility which causes phytotoxicity to plants (Everhart, 1994). Plants vary in their tolerance and response to Al and Mn toxicity, indicating a crop-specific soil pH requirement. While soil acidification involves a purely chemical reaction, biological association in the form of soil microorganism must metabolize those fertilizers before effectively lowering the soil pH. Therefore, lowering soil pH requires the proper soil properties and conditions suitable for microorganisms to assess the biological reaction. (McCauley et al., 2009)

### 8.3 Soil pH buffering capacity.

One of the fundamental soil properties is its pH buffering capacity (pHBC) (Bloom, 2000). Soil pHBC has been used to estimate the change in the soil pH after acidic or alkaline elements are added to the soil. Quick and accurate determination of

soil pHBC if achieved at low cost can be used to assess the agricultural liming or sulfur recommendations (Liu et al., 2004). It can also serve as a long-term predictor for the rate of soil acidification by knowing previous external acidity sources.

Nitrogen simulation models such as mineralization, nitrification, urea hydrolysis, $NH^3$ retention, and volatilization require knowledge of soil pHBC (Kissel et al., 2012). These N cycle reaction rates and chemical speciation depend on soil pH and consequentially depend on soil's pHBC along with other environmental factors. The typical determination of soil pHBC is done using multiple doses of the base in a titration procedure to construct a pH buffer curve (Nelson and Su, 2010). The titration curve of the topsoil is usually linear in the pH range of 4.5 -6.5 (Magdoff and Bartlett, 1985). The inverse of the slope of pH to the amount of base added to the soil is defined as pHBC and is expressed in millimoles $H^+$ per kilogram per pH unite (Kissel et al., 2012). From the titration curve, the soil pH increase can be estimated from the millimoles of $H^+$ used to make a change in the pH per kilogram of soil. The titration process can also be used to estimate the soil pH decrease from the millimoles of $H^+$ added per kilogram of soil. Then the change in the soil pH (ΔpH) can be predicted from either the addition or removal of H+ (ΔH+, mmol H+ kg−1) from the soil as

$$\Delta\text{pH} = \Delta\text{H} + \frac{\Delta\text{H} +}{\text{soil pHBC}}$$

The titration requires a lengthy procedure of many days of incubation to reach a pH equilibrium. It requires a wide range of solution /soil ratios and different solution ionic strengths. Atken and Moody suggested that the soil pHBC is a fixed soil property but it is still unclear when soil pHBC values form the same soil are different due to

different bases like; $Ca(OH)^2$ vs. NaOH or acids HCl or $H_2SO_4$ (Kissel et al., 2012). This discrepancy is due to the sensitivity of soil pH measurements to the changes in ionic strength of a solution with electrical conductivity (EC) less than 2dSm $^{-1}$ (Miller and Kissel, 2010). However, this condition can be avoided by using a uniform ionic strength for titration, organic ionic strength or to process the titration in a low salt solution (< 0.01 $molL^{-1}$). Nevertheless, Calcium chloride ($CaCl_2$) is the best option in most cases because $Ca^{+2}$ is normally the dominant exchangeable cation in arable surface soils (Kissel et al., 2012). Thompson et al. (2010) compared the time required to raised several soils pH using $CaCl_2$ and found that it took 4 incubation days to increase average pH by .023 (). Another study reported that the incubation of acid soil with base required substantial time to reach equilibrium pH (Aitken and Moody (1994)).

### 8.4 Pot-in-pot for fruit tree plantation

Planting fruit trees directly in a nonhomogenous soil is not the best option for growing plants is the cheapest option (Erez et al., 1989). Even with long historical use of soil analysis, the soil is still a mystery in term of all physical, chemical and biological complexity that might occur. Thus, container growing present not only an aesthetic and luxurious way of growing plants but also a tool for researches to evaluate certain aspects that might influence plant growth and productivity. It relieves the plants from two major constraints, the unpredicted climate and the unknown soil dynamics (Burdett et al., 1983).

The large number of quantitative studies designed to determine the effect of a certain factor affecting fruit tree have been done using potting methods (Marsal et al., 2000). This approach maintains almost full control of other environmental conditions

that might act as confounding factors by providing uniform soil variables. Those studies included the investigation effect of irrigation on pears fruit (Marsal et al., 2000), root restriction of apple and peach trees (Myers, 1992), effect of root pruning on apple trees (Hsu et al., 1996), nitrate absorption by orange trees (Chapman and Parker, 1942) and evaluation of chemical control of pathogenic disease in apple (Kirby and Frick, 1963).

Hoestra studied the effect of soil pH on apple seedlings in pot experiments and found a good growth of apple seedlings at pH 3.8 (Hoestra, 1968). When using this approach, it is essential to pre-adjust soil pH and to maintain consistency throughout the experiment. Root temperature can cause a dramatic change in the root growth and ultimately in overall tree growth. Direct sunlight can be absorbed on the exposed surface of the container leading to significant temperature fluctuation and extreme temperature during the summer (Martin and Ingram, 1992) and the winter (Mathers, 2003). To avoid this problem, the trenched pot-in-pot system can be used. where pots are stacked and placed in 80cm wide x 70cm deep trench.

# 9 Oman prospective.

## 9.1 Al Jabal Al Akhdar

The Sultanate of Oman is located in the Arabian Peninsula between latitudes 16° and 28° N, and longitudes 52° and 60° E where the climate is favorable for arid and semi-arid plants. However, the region of the Al-Jabal Al-Akhdar, an Arabic translation of the Green Mountain, has a Mediterranean-like climate supporting the growth of temperate trees. Al-Jabal Al-Akhdar is part of the Hajar Mountains that was described as a local center of plant endemism (Miller and Nyberg, 1991). This plateau raised at an altitude of 3,000 meters above sea level (23.07 N, 57.66 E) and is the location of around 33% of Oman's 1200 species of vascular plants, from 14 taxa that are endemic to Oman

(Brinkmann et al., 2009).

The first report in English describing the location of Al-Jabal Al-Akhdar and the type of vegetation grown there was written as part of the historical section of the British Foreign Office titled “Persian Gulf” in June 1919 (Anonymous, 1919).

### 9.2 The climate and the vegetation.

The climatic condition is characterized by lower temperatures at which the winter minimum temperatures satisfy the chilling requirements of many deciduous fruit trees that have been cultivated in Al-Jabal Al-Akhdar such as; [apple (*Malus* × *domestica* Borkh), pear (*Pyrus communis* L.), sweet cherry (*Prunus avium* L.), apricot (*Prunus armeniaca*), peach (*Prunus persica)*, plum (*Prunus domestica*), table grape (*Vitis vinifera*), pomegranate (*Punica granatum*. L)], nut species [almond (*Prunus dulcis*), pistachio (*Pistacia vera*), walnut (*Juglans regia* L.), Olive (*Olea europaea*) and roses (*Rosa* × *damascena*)].

Local farmers have been growing native temperate fruits for hundreds of years and used primitive tools to prepare the field for planting. They used complex gravity-driven open water channels for irrigation fed by springs (Figure2,c,d). However, in the last 20 years, machinery and bulldozers have been implemented to establish fields for fruit trees production and modern irrigation systems. Growers are also trying other fruit trees and cultivars that have not been cultivated before like chestnut (*Castanea dentata),* blackberries (*Rubus fruticosus* L.) and raspberry (*Rubus idaeus*).

### 9.3 The geography

The Al-Jabal Al-Akhdar area is made up mainly of highly permeable carbonates (black limestone and brown dolomites) laying on rocks of the pre-Late Permian Sedimentary Basement, conformably overlain by the Mahil Formation (Be´chennec et

al., 1992). Rocks are generally exposed, steep, and with a thin layer of soil and sporadically covered with some vegetation that is primarily found in grooves or small depressions with an accumulation of sediments between stone cracks (Figure.2b). Large rocks, small stones, and mixed gravel can be found in the steep grooves. However, downhill wadis contain mainly gravel and accumulations of sandy soil mixed with sedimentations. The infrequent rainfalls in winter can lead to flash floods that rush through the barren valleys (Brinkmann et al., 2009). The average annual precipitation at Al-Jabal Al-Akhdar ranges between 100 and 340 mm with a higher chance of rainfall from February–March, and from July–October (Luedeling and Buerkert, 2008).

### 9.4 Potential for apple expansion in Oman

A Ph.D. project by Luedeling (2007) on sustainability of Al-Jabal Al-Akhdar oases analyzed 24 years of metrological data, topographic maps, and digital elevation model (DEM) along with irrigation water hydrology. The study showed that the climate and the chilling hours still sustain growing temperate fruit tree on higher elevation villages (> 1800m) like; Ash Sharayjah, Al ‘Ayn, Al ‘Aqar (Figure 2).

Despite the favorable climate condition, the author found decreases in temperate fruit tree farming due to demographic changes, lack of irrigation water and obstacles in sustaining profitable farming in that soil conditions. However, one of the main constraints for expanding apple production is the high alkaline soil pH. In this region, soil parental materials are weathered limestone (Koehrer et al., 2010) in which calcium carbonate is abundant raising the soil pH to medium alkaline. In many other apples growing area around the world, extreme pH values are a limiting factor for apple production. In Oman, growers either bear burdens of expensive transport of near-neutral

soil to grow fruit trees or depending on the native seedlings which are adapted to the high pH.

Those native domesticated cultivars have low yield and poor quality and are susceptible to many local pest and diseases. This is because of lack of resistance in their genetics and the higher soil pH hinders the uptake of many soil elemental nutrients which consequently reducing their vigor and defense mechanisms. The diverse genetic background of apple has been very useful to apple scion and rootstock breeders. An evaluation of rootstock tolerance to soil pH should provide rootstock breeder with information on their adaptability and performance to low or high soil's pH conditions that have not yet been fully characterized. Nevertheless, identifying the proper rootstocks that tolerate extreme soil pH and improving nutrient uptake capacity along with adaptaion to low chilling areas would also be necessary.

If adapted rootstocks can be identified, It will encourage Omani growers to grow improved and adapted rootstocks to their orchards conditions as well as improving their productivity while lowering the field horticultural practice resulting in profitable production. Hence this study sought to evaluate several rootstocks before making a recommendation to be introduced to Oman and other apple's growing area with similar extreme soil pH.

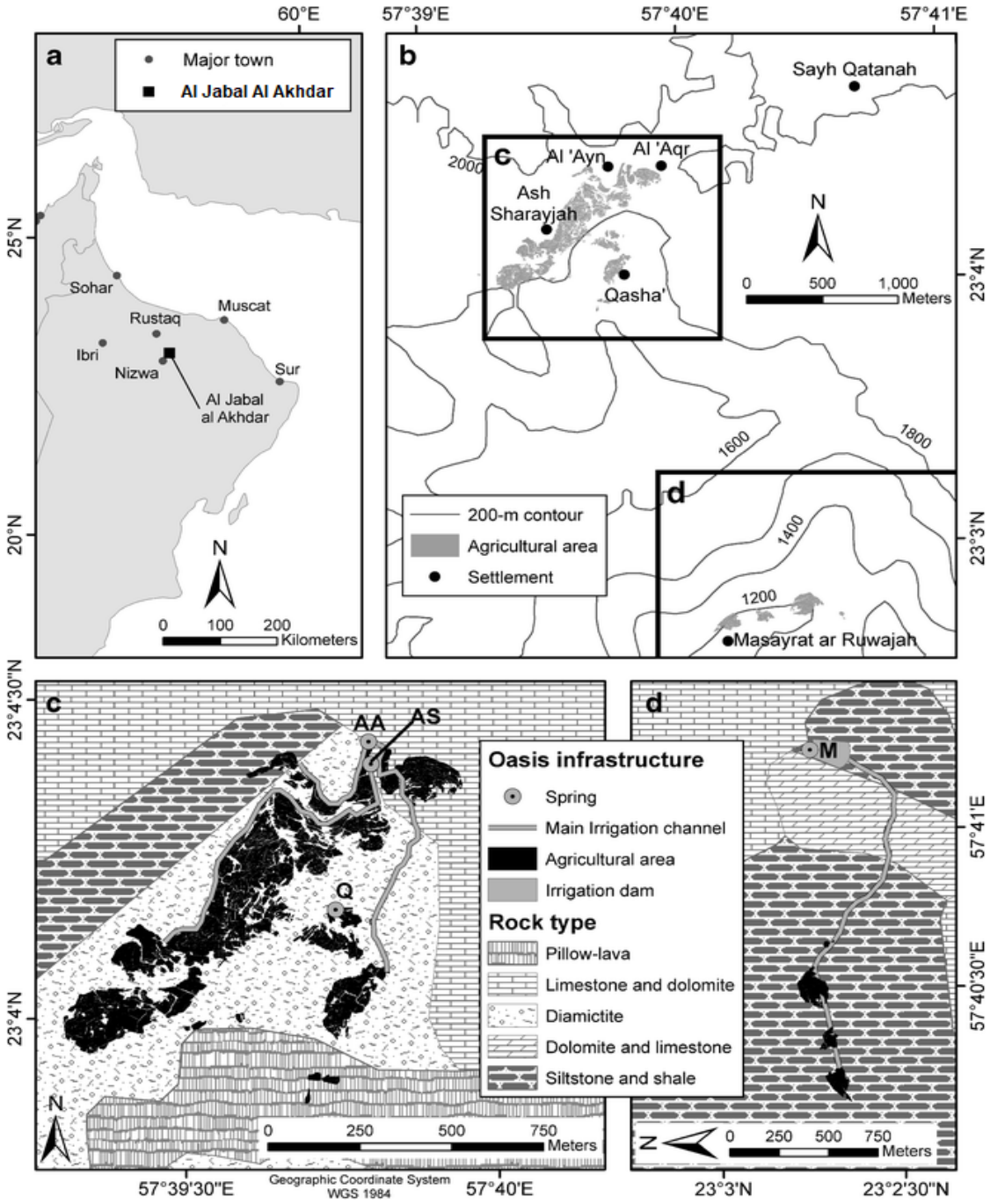


Figure 3.Location of Al Jabal Al Akhdar region in Oman (a), inhabitant villages and their topographic altitude (b), geologic component of the higher altitude agricultural areas (c) geologic component of the lower altitude agricultural areas (d). AA, AS, Q shows the water springs and irrigation channels. (Luedeling and Buerkert, 2008).

## 10 Plant's root system

When Charles Darwin concluded his book the 'Power of Movement in Plants' proposing a root theory by stating: "It is hardly an exaggeration to say that the tip of the radicle thus endowed and having the power of directing the movements of the adjoining parts, acts like the brain of one of the lower animals; the brain being seated within the anterior end of the body, receiving impressions from the sense-organs, and directing the several movements." It was clearly implied that Darwin highlighted the significance of the root tip as the brain organ that uses its sensitivity to navigate and scout for nutrients within cracks of the soil. He also signifies the importance of less studied plant's part yet a vital organ (Baluška et al., 2009).

### 10.1 Fine and lateral branching root production

Fine roots are the most active and dynamic part of the root system. They play an important role in scouting and navigating below ground medium for water and nutrients as well as performing nutrients uptake (Artacho and Bonomelli, 2016). Fine roots have been well described and characterized as a short-lived, non-woody and very small in diameter of < 2mm varying within fruit tree species. They are considered an important and dynamic component of all terrestrial ecosystems (Ma et al., 2013). The continuous production (initiation and mortality) of the fine root throughout the growing season makes a variance in fine root's ages, length, mass, diameters, color, branching order and architecture (Wells and Eissenstat, 2002).

When fine roots first emerge, their color is white to pale white expressing rapid elongation and extension. This is due to the high capacity of water and nutrient uptake but as they grow older, the epidermis turns brown with a decline in nutrients uptake and respiration (Comas et al., 2000). However, another study found that older root of tree

seedlings significantly provide higher contents of nutrient uptake due to bigger root surface area (Hawkins et al., 2014).

Evaluation of fine root's length was found to provide a better index in determining root production and turnover when compared with other root indicators (M. G. Johnson et al., 2001).

### 10.2 Fine root categories.

Plant's roots were classified into four major groups depending on the initiation organ the developed from; the seed, the shoot, the hypo/mesocotyl, or other roots (Zobel and Waisel, 2010). The lateral roots class can be found in most of the plant specious and it is made up of most of the root length. However, the root weight is not considered within the lateral roots class due to its smaller diameter. The formation of lateral root primordia is the starting point for the development of the later roots. This is happening just behind the root tip of the main root. These primordia go through 9 different steps of which the last step is when it emerges from the cortex of the main root just behind the elongation zone (Postma et al., 2014).

This rapid sequence of development happens in just days after the first cell divisions which prime to their formation (Malamy and Benfey, 1997). During these developments stages, some primordia will remain dormant while others will develop into lateral roots (Dubrovsky et al. 2006). The rate of primordia formation determines the final number of lateral roots. Many studies have been investigating the correlation between the primordia formation process and lateral root emergence, (Lavenus et al., 2013) and the association with some genes activation (Caboni et al., 1997) during the different steps of regulating hormones (López-Bucio et al., 2003).

### 10.3 Functions and formation of the lateral roots.

It is well understood that the formation of lateral roots increases the anchorage strength of the root system and eventually promoting the development of longer root length. This ultimately leads to better soil's nutrient and water acquirement. Conversely, a higher lateral root branching density (LRBD) makes roots denser which leads to rise in competition for nutrients and water within the same plant's roots. Hence negatively plummeting the uptake efficiency per unit of root length and could be modeled as decrease root efficiency when root system increases in size (Berntson 1994). Figure 1 compares a computer-generated model of high (LRBR) with low (LRBR) (Postma et al., 2014). The study found there is a metabolic cost in producing additional root length in high LRBD situation. They found the growth of other roots or the shoot will be reduced due to the consumption of metabolites in construction and maintenance of the additional root length. This cost of metabolic consumption is either calculated in units of carbon or in terms of other used limiting resources (Lynch, 2007b). Consequently, the balance of the marginal cost of root production and the marginal utility of soil resource acquisition determine the optimum number of lateral roots.

Therefore, environmental conditions also contribute to the optimal LRBD in a given ecosystem (Postma et al., 2014). However, still, no obvious values can be found in literature quantifying the optimal branching density and how different environmental conditions influence the optimum lateral branches per centimeter of parent root.

Since the availability of soil resources are persistent limitations to plant growth, studying the cost and tradeoffs of LRBD present a better understanding of the root architecture dynamic and plant adaptation to environmental conditions. These

parameters would serve as a selection tool for trait-based to breed cultivars with high productivity and adaptability to suboptimal nutrients soils. (Lynch, 2011, 2007)

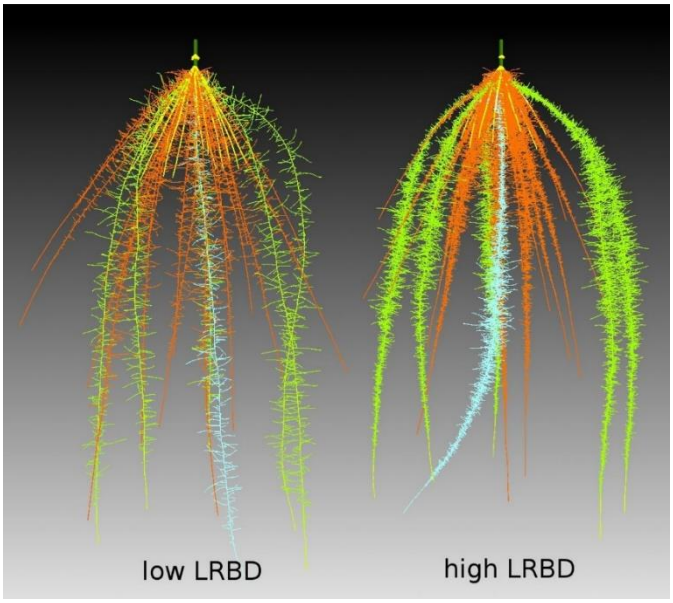


Figure 3. Computer generated image of two simulated maize root systems. The model presents 40-d-old maize root systems with 2 (left) and 20 (right) branches cm−1 major root axes. Postma et al., 2014

### 10.4 Root's lifespan

The end of the root's life, defined as replacement phase or turnover, varies among trees from (0.4-2.8 year -1) which explain the short lifespan of the fine root (King et al. 2002). There is limited information about the turnover rate in fruit tree (Artacho and Bonomelli 2016). However, several studies estimated this rate by calculating the median of 50% mortality of root population and showed that it varies from 30- 100 days (Bouma et al. 2001, Wells and Eissenstat 2001). This could explain how fine root that emerges each season will subsequently undergo senescence and turn brown just prior to the end of their first growing season. (Artacho and Bonomelli 2016).

The lifespan of fine root and the ultimately turnover rate varies among tree species and can be influenced by many factors. One of these factors soil temperature. Studies

found as soil temperature increases, the root lifespan decreases (Pregitzer et al., 2000). Other aspects include nutrient availability and soil water content contribute either positively or negatively to root lifespan depend on measuring methodology, plant species, and the rhizosphere environment. Nutrient availability is a contradictory hypothesis with inconsistency results when it came to assessing the effect of nitrogen availability in the soil to root lifespan (Artacho and Bonomelli 2016). Some studies show a negative effect on root lifespan in rich-nitrogen soil in Pine tree species (Johnson et al. 2001). Other stated the opposite and showed that N availability decreases fine root turnover rate (Jia et al. 2010) or no effect to lifespan (Rytter 2013). A study on the effect of apple replant disease (ARD) on the growth rate of the fine root in apple rootstocks shows that differences in root growth and the turnover rate are due to differences in the cost of tissue infection and necrosis to the plant (Atucha et al., 2014). They reported that total root biomass of root ratio was higher in the tolerant to ARD rootstock (CG.6210) than in susceptible rootstock (M.26) when grown in soil from an apple replant disease location. Roots of CG.6210 were thinner and had lower N concentration (Atucha et al., 2014). High rootstock growth rates and rapid root initiation has been associated with tolerance to leaf galls caused by phylloxera on grape rootstocks (Bauerle et al., 2007). Early turnover of root browning and the accelerated browning rate is a sign of root response to many factors including low soil moisture and high soil temperature (Anderson et al., 2003), below root pest and disease interaction (Liljeroth, 1995) (Liljeroth1995).

Root pigmentation undergoes through changes as an indication of the senescence of the root cortex and associated with other physiological changes that lead to decrease in

nutrient and water uptake, and respiration rates (Wells and Eissenstat2002).

### 10.5 Root-rhizosphere interaction.

There are four main environmental factors affecting the root system development; moisture, temperature, mineral concentrations, and gaseous atmospheres (primarily [CO2] and [O2]) (Zobel 1989, Russell 1977). Root phenology was found to be affected by exogenous factors like soil temperature, moisture and nutrients availability (Noguchi et al., 2013). Several studies show that root phenology is more affected by soil temperature rather than soil water content or nitrogen availability (Majdi and Öhrvik 2004, Steinaker et al. 2010, Fukuzawa et al. 2013, Steinaker et al., 2009). Other explained the pattern of root production in fruit trees as wide variance from unimodal and bimodal to normal distribution. (Eissenstat et al. 2006). Fine root follows a time growth pattern and undergoes phenological changes in response to internal and external influences.

### 10.6 Response to endogenous factors:

Effects of internal factors vary among tree species similar to the response of external aspects related to environmental condition and rhizosphere (Fig.2). Other findings supported the response of root development by the influence of rootstock genotype (Atkinson and Wilson 1980, van Hooijdonk et al. 2011), age (Wu et al. 2012), orchard management (Eissenstat and Duncan 1992), crop load (Rosecrance et al. 1996) and plant density (Atkinson and Wilson 1980). Other contributors to root phenology are endogenous such as carbohydrate utilization from source to sink and plants hormones

(Berman and Dejong, 2003). A study showed that fruit trees are mainly affected by source-sink relationship due to the development of fruit that presents a stronger sink to carbohydrates (Grossman and DeJong 1995). Several studies showed that plant's root

growth was declined to a minimum during maximum fruit growth (Inglese et al. 2002, Mimoun and DeJong 2006, Basile et al. 2007, Abrisqueta et al. 2008). Moreover, the response of source-sink to root's development also linked to the relation between root-shoot growth in woody plants (Steinaker et al. 2010). Particularly in the apple tree, it was reported that the highest fine root growth was recorded a few days or weeks after shoot extension. (Psarras et al. 2000, Wells and Eissenstat 2001).

### 10.7 Response to exogenous factors:

Several environmental factors affecting roots growth and expansions. Those are; moisture, temperature, mineral concentrations, and gaseous atmospheres.

**Moisture**

Moisture is a vital factor for plant and root survival. It is involved in moving water from the soil to the root system and dispersal of the root system to follow moisture in the soil. For example, irrigation and rainfall frequency determine the distribution of the root system from deep in the soil root in infrequent soil moisture to shallow root in more frequent moisture conditions (Carmi 1986). Studying the water-root system interaction in- vitro has been very complicated since the available system utilizes pot plantation or hydroponics. Both methods do not mimic normal field growth due to either root restriction within pot size or excess moisture in hydroponics (Torrey and Winship 2012).

**Soil Temperature**

Many studies investigated the effect of soil temperature on root development in extensive research, but the correlation is still not fully understood. Rhizosphere's temperature is often seeming linked to decline in root growth. This causes decreasing in root penetration into soil profile in low-temperature conditions which leads to increased

plant's water deficit in the dry season. This also will decrease the overall plant growth as well as the root-to-shoot ratio leading to higher photosynthate amount utilized by root (Cooper 1973). Earlier studies reported the first evidence on a measurable effect of temperature to crop growth as a small difference as 1°C (Walker 1969). Studies adopting this hypothesis have been using an alternative growing system to allow non-destructive assessment of all parts of the root system in control temperature and optimum root zone and shoot environments (Torrey and Winship, 1989).

**Mineral nutrition**

A soilless growing system such as hydroponics and aeroponics provides opportunities to study the minerals nutrition of plants has the advantage of controlling and monitoring mineral solution and nutrients uptake (Bloom et al 1992). Observing the effects of minerals solution on the plant's overall growth and root development precisely was accurate and precise under the aeroponics system where all other limiting factors were controlled. Several studies observed the multidirectional effects of temperature, gaseous atmospheres, and moisture on plant's growth and specifically on the root growth and physiology. Thus, a good assessment's design to mimic field-like plant mineral nutrition is necessary to maintain control of all aspect of the rhizosphere environmental (Torrey and Winship 2012).

### 10.8 Nutrients uptake in response to varying pH levels.

Several studies have shown that the plant's root is capable of changing the rhizosphere pH. This consequence is also responsible for root respiration, the release of $CO_2$ and disproportion of cation uptake (Aguilar, S. and van Diest, 1981). These changes in pH can be measured in the nutrient solution to understand root uptake and response in overall plant growth (Marschner and Romheld 1983). Generally, pH

changes at the soil-root rhizosphere due to cation-anion uptake. However, the pH buffering capacity of soils might respond to these root-induced changes by limiting them to a narrow zone adjacent to the root surface.

pH and EC play an important role in plant productivity grown conventionally or in a soilless system due to their association with nutrients uptake. The pH level and EC concentration of the nutrient solution in the aeroponics system affect the availability of nutrients (Asao 2012). The nutrient's availability varies depending on mineral solubility on acid or alkaline medium as well as among ion concentration (Borgognone *et al* 2013, Friedman 2005). Thus, it is very important to control the pH level and EC concentration to prevent hindered growth due to absorption implication. However, root exudates influence changes in the rhizosphere pH which is responsible for nutrients dynamics and ultimately the availability of mineral nutrients for plants. Earlier studies in plant's nutrients dynamics were reported to be reduced by root-induced pH. This clearly implies that roots can influence both solubility and mobility of nutrients.

The concentration of the micronutrients Mn, Fe, Zn, and Cu in the soil solution primarily can be subjected to changes in soil pH, redox potential, soil organic matter content, and temperate (Marschner and Rengel, 2012). Low pH or redox potential, can induce an increase in the availability and concentration of Mn, Fe, Zn and Cu (Sims and Patrick, 1978; Miao et al., 2006). Gaudin *et al* 2011 conducted an experiment in maize grown in aeroponics system and verified that low nitrate intensely increased the crown root elongation and decrease in crown root density and reduced root hair length and density. However, this low nitrate situation leads to an increase in the density and length

of the lateral root.

## 11 Apple root architecture:

The most well-investigated part of the apple tree has been their rootstock where many studies evaluate rootstock dwarfness (Auvil et al., 2011; Ferree et al., 1995; Foster et al., 2017), precocity (Carlson, 1975; Webster, 1995), disease resistance (Jensen et al., 2012b; Robinson et al., 2003; Russo et al., 2007b), crop load (Adams et al., 2018b; Albacete et al., 2015; Robinson et al., 2011), water and nutrients uptake (Blok et al., 2017; Cheng and Raba, 2009; Schupp, 1995), high density production system (Fallahi et al., 1984; Robinson, 2008), anchorage (Fan and Yang, 2011), suckering (Adams et al., 2018b). However, many root's characteristics and functions of those rootstocks are still mysterious. These include; root distribution and fine root production patterns (Artacho and Bonomelli 2016), root architecture (Weiguo Fan and Hongqiang Yang 2008), root-soil interaction (Hinsinger et al. 2003) root-root interaction (Faget et al. 2013).

Apple rootstock is one of the fascinating parts of the apple tree where no other above-ground crop is devoted to roots like in apple's tree (Gardening. Cornell factsheets). Though, still limited information available due to imperfect methods available to conduct experiments for evaluating and monitoring root development in a non-destructive way.

The term root architecture is not less important than tree architecture when it came to providing tree stability, supporting nutrient and water efficiency and help in high yield of fruit quality. Root architecture refers to the spatial configuration and distribution of the root system in the growing medium. Its significant function in any

root system came from the efficacy of water and nutrients uptake from the soil.

A study on apple rootstocks (*Malus hupehensis* Rehd.) found that lateral root numbers, densities, and lengths declined when grown in sandy soil but increased in clay soil. Fan and Yang, 2008 reported that apple lateral roots from the primary root were found in a good distribution pattern in sandy soil. However, lateral roots were dense on the upper part of primary roots when grown in clay soil. They noted that adding organic matter and fertilizer would reduce the lateral root's lengths and numbers in clay soil, while increased when compared to sandy soil. On their study of the effect of soil condition on apple root architecture, they concluded that a shift in root architecture from well-distributed lateral roots on the primary root to clustered lateral roots on the upper portion of primary root was based on soil particles and texture (Fan and Yang, 2008).

Plants with a great complex root architecture benefited from a greater interface with the soil and ultimately a greater absorption potential (Locatelli et al 2002). This massive network of root's segmentation and production required a big utilization of carbon to grow and maintain an active root's function (Fitter et al., 1991). The initiation of root undergoes a progressive stage starting by production followed by elongation and branching (Pellerin & Pagès, 1994).

Most studies on root experiments have been expressing the root's growth in terms of total length or mass and overlooking factors affecting root development by describing them as variation measures. Though, only considering length and mass could lead to inaccurate conclusion due to the missing measurement of carbon dynamic allocation. From such studies, a poor correlation is presented between root mass and absorption capacity due to setting their main axis to biomass and in this analysis, it

works only in uptake (Locatelli et al 2002). At this point, the importance of assessing root architecture arises as the root system with high lateral root has higher water and nutrients uptake capacity compare assessing just the root biomass (Hetrick, 1991). Regardless of this misinterpretation, limited studies highlighted the importance of root architecture characteristics listed below:

Generally, studies on root architecture use a combination of imaging and mathematical models. Some of root architecture parameters that are generated by root images analysis software include; number of roots, ellipse axis, Network area, Network bushiness, Network convex area, Network depth, Network length, Network length distribution, Network perimeter, Network surface area, Network volume, Network width, Network width to depth ratio, Number of connected components.

An order number is assigned to roots based on connectivity to the stem (zero-order if the radicular axis is connected to the stem and [one] if subordinate root connected to order zero) (Bernston, 1997). The importance of understanding the root order differences came from variance in root functions, growth patterns, longevity and structural features that have a direct impact on root absorption capacity (Hooker & Atkinson, 1992). Researchers found an obvious association between the root architecture and nutrients use efficiency which shapes the root distribution but understanding the genetic basis of the root system will provide a better phenology understanding (Fan and Yang, 2011; Lynch, 1995; Pagès and Pellerin, 1994).

### 11.1 Root's architecture and development

The uniqueness of plant's roots is their ability to effectively forage for water and nutrients even under stressed environment and unfavorable condition for plant growth

(McCully 1995). The morphology and physiology of plant roots determine the capacity of mineral uptake due to the higher ratio of surface area to volume of soil (Lynch 1995). Overall, root system contributes as a community of individuals by bringing varying efforts in navigating and absorbing water and nutrients depending on their individual features, order, age, and location (Volder *et al*. 2005). Similar to plants grown in the soil, those grown in aeroponics system vary in the amount of water and nutrients uptake. However, in the aeroponics, the volume of nutrients solution in contact with roots will determine the root morphology and architecture. The volume of nutrient solution limits the amount of branching and distance to which the root extends horizontally and vertically (He, 2009; Qin et al., 2007; Tan et al., 2002). Plant roots can alter their nutrient acquisition capacity by dynamically adjusting their initiation, morphological, architectural and/or physiological characteristics to supply shoot nutrient demand according to rhizosphere environmental stress (Forde and Clarkson, 1999).

Some studies demonstrate the importance of root architecture and the influence of root's distribution on nutrient uptake efficiency while others explain that the characteristics of long lateral roots may help plants to tolerate water stress (O'Toole and Bland, 1987). In contrast, others conclude their experiments on root density and branching with no significant effect of root architecture on water uptake when testing both monocotyledonous and dicotyledonous plants (Petrie et al., 1992).

Root initiation implicates series of formation of root meristem from continuous cell divisions of induces cells. The formation of root meristem also undergoes complex changes in the metabolism which include the interaction of many factors leading to the development of adventitious root formation (Caboni et al.1997).

## 11.2 Root formation.

Root development is highly associated with the plant's growth and development as well as adaptation to the surrounding environment and soil's properties (Figure 5.)

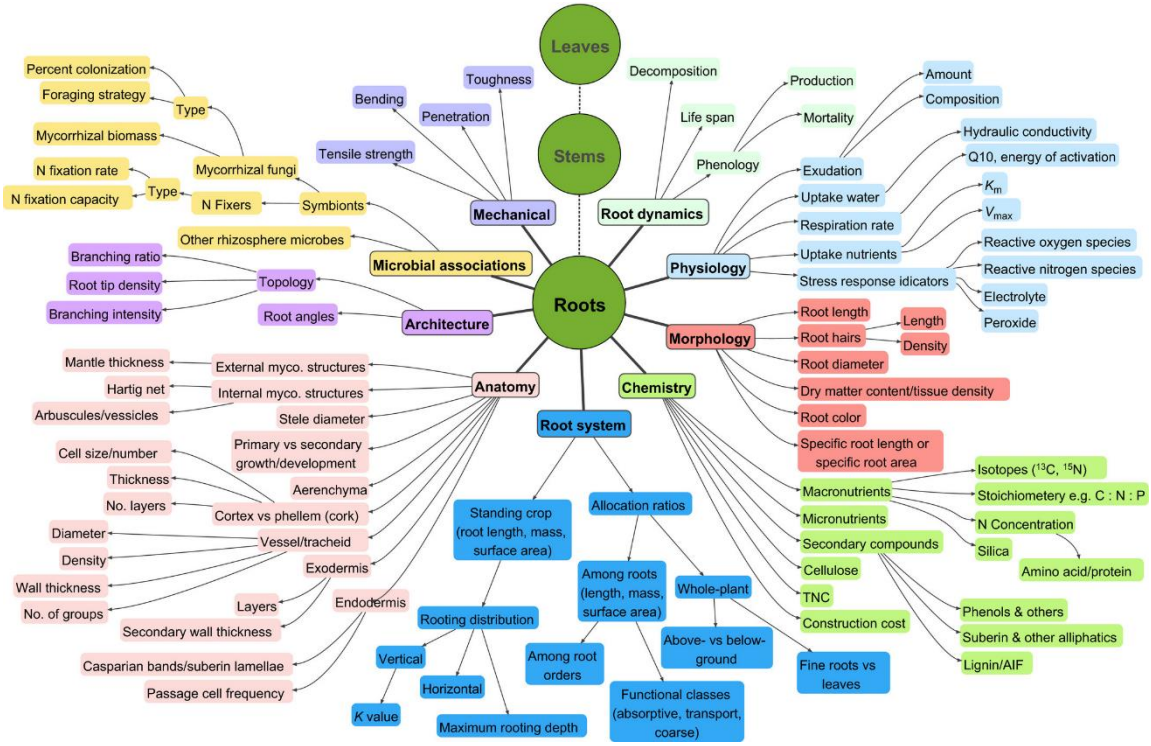


Figure 5. Map of fine root's functioning traits. Traits are grouped into anatomy, architecture, chemistry, mechanical, morphology, physiology, root dynamics, microbial associations, and root system. (McCormack et al 2017.

The root's size and distribution are well-known factors for their main role in nutrient uptake. (Fitter 1991). Research on root's growth could correlate the effect of root architecture on the nutrient uptake from the rhizosphere. (Barber and Silberbush (1984) Itoh and Barber (1983).

One of the unique characteristics of plants' root is the ability to develop repetitive branching into distinctive angles and axes. The root's structure is composed of endless repeated formation of axes representing the vegetative phase of the plant's growth. These formations are referred to as adventitious roots where they play a vital role in nutrients uptake, plant growth, development, and the overall plant's life cycle. It

consists of a range of root's shots emerging from the already formed root and formed the root architecture. (Peter W Barlow and Beatriz Palma. The place of the root in plant development). The definition of the term 'adventitious root' is a formation of small fine roots by artificial means with the assistance of plant growth regulators. (Avery et al.1947, Blazich 1988). The adventitious term explains the anatomically unexpected position where the roots initiated from the root primordia. (P. W. Barlow and B. Palma).

### 11.3 Adventitious root classification

The initiation may be a response to external or internal stimulants or both that caused root emergence. Furthermore, the position and timing of root initiation could be either from a pre-formed or a post-formed spot that was regulated during cell division and fragmentation. This term includes various categories of root types with different anatomical origins controlled by organo-genetic circumstances. Barlow and Palma classified roots into eight categories based on initiation point, prediction and influence by other stimulants (Barlow and Palma, 1997). Their eight categories of root considered to be the first descriptive of adventitious roots:

I. Sylleptic shoot-borne root

2. Proleptic shoot-borne root

3. Sylleptic, adventitious shoot-borne root

4. Proleptic, adventitious shoot-borne root

5. Sylleptic, adventive shoot-borne root

6. Proleptic, adventive shoot-borne root

7. Induced, adventitious shoot-borne root

8. Induced, proleptic, adventitious shoot-borne root.

Considering the above eight root categories, the emergence root's site is predictable in root type 1,2,5 and 6 whereas in all other types root site are infrequent. Barlow and Palma associate their root descriptive categories to time and position of the root's origin but stated that embryonic root is still unresolved confusion to which of the eight groups can be categorized. They further elaborate the root's architecture of Gramineae and Equisitaceae as a result of a photometric unit that explained the self-directed sectional of plant growth but not evidence in dicotyledonous.

### 11.4 Dynamic of root architecture.

Another significant feature of roots, specifically in trees species, is that trees stabilize anchorage through transferring the loading force from the stem into the ground and subsequently to the root. This load will ultimately shape the root system based on how the load force is distributed. In the situation of the large root, surface area, the force will rapidly degenerate. This can be achieved by either larger or highly branched roots. Similar root dynamic can be observed when the tree is subjected to adverse wind condition (Stokes and Guitard, 1997). A follow-up study reported response changes in the root system in term of lateral root number and their orientation (Stokes et al., 1995). These changes in root architecture can influence tree stability and overall anchorage. Other environmental changes such as soil temperature, moisture and $CO_2$ can reflect in the dynamic response of the root system. In contrast, the higher air temperature will limit shoot or fruit development at which root growth will flourish.

Another finding reported that the root distribution of young apple trees differs from older trees (Hughes and Gandar, 1993). While young trees follow a bowl-shaped with roots centered near the stem, older trees have a more layered structure with higher root

length density distributed further away from the trunk. Similar findings were reported by (De Silva et al., 1999). The annual root growth dynamics also vary based on the tree's age.

### 11.5 Dynamic of apple root architecture

A specific study on apple rootstock M.9 found five pattern peaks in root growth during one growing season and correlated it to the effect of climate factors, soil properties and rootstock-scion interaction (Psarras et al. 2000).

Another study on older Golden Delicious apple trees shows three peaks of growth (Ma et al., 2013) compared to a single peak of annual root growth on potted young apple trees (Wang et al.1997). These findings and other support the importance of continuous monitoring of the root system for better understanding of root dynamics and architecture. It can be concluded by supporting (Reddy 1997) statement in linking the changes in the rate of the root growth that eventually affect the root distribution in the soil profile and consequently water and nutrient uptake.

## 12 The history of Aeroponics:

The plant cultivation process requires a basic supply of water, nutrients, air, and light to initiate and sustain growth. The modern agricultural techniques were able to control these environmental factors and optimized them to flourish plant growth and productivity (Nir I 1982). Researchers were faced with many challenges to understand the plant- environment- soil interaction until the first discovery of the basic mineral nutrients that essential for plant growth and development. The experiments on first synthetic fertilizers by Liebig (1803-1873) and Businago (1802-1887) followed by Hoagland (1938) stir researches toward the ability to grow the plant in only nutrients

solution. Since then, many types of controlled soil or soil-less medium that can provide nutrients and water for the plant to maximize their growth and productivity or to reduce labor and land cost. Many growing systems were improved and optimized for a specific production system or to be used in research programs.

The earlier work by Hoagland and Arnon in 1938 in hydroponics methods was a breakthrough in advance agriculture at that time. This technique was first tested to examine suspended root in the air by Carter 1942, Went 1967 and Zobel et al 1976). Historically, aeroponics or misting chamber as used to be called was used also to study the root and rhizomes in a non-destructive way (Koller and Nir 1972). It has been used as a tool to study root physiology (Barker 1922) and it was reported to be a reliable technique for stable control of nutrients, oxygen, moisture and root ambient temperature (Zobel 1989).

## 12.1 Aeroponics System: [Technical details]

Aeroponics can be defined as a soil-less plant culture where fresh nutrients solution is timely regulated to continuously supply a mist to suspended root inside a sealed container in a dark environment. (Nir 1982.; Engenhart 1984; Zsoldos et al. 1987; Barak et al. 1996; Mbiyu et al. 2012). Farran and Mingo-Castel (2006) explained the nomenclature of terms Hydroponic and Aeroponics was adopted from the Greek and Latina terms Hydro and Aero meaning water and air and Ponic means labor. In those systems, plants are grown in a soilless setup while supported by a constructed structure in a controlled environment. The plant roots grow either suspended in the air under misting nutrients or wholly immersed in nutrient rich-water (Beibel 1960; Reyesa et al. 2012). Thus, to obtain a reliable result during a biological study on roots, it needs a precise control of all factors contributing to the root zone environment. Scientists studying root dynamics depending on various soilless culture system and the aeroponics can provide them with the optimum control of the rhizosphere (Weathers and Zobel, 1992). US Patent Publication No 1999/5937575A defined the aeroponics as a cultivation growing system providing advantages for agricultural scientific research and production as advance experimental tool (Lakhiar et al 2018).

### Components & Mechanism

Hydroponics and aeroponics have been extensively used in studies to understand plant growth and interaction in a controlled environment. In Hydroponics system, plants are grown in the suspension of nutrients with a continuous flow of nutrients dissolved in water. During this process, water, nutrients are mixed to provide roots with balanced growth essentials. Due to improper aeration in hydroponics systems, aeroponics system

was developed to solve that problem and to provide an adequate air source for root respiration. In aeroponics, nutrients solution is mixed with water and air while spraying directly to the plant's root. This system consists of pumps, tanks, sensors, and regulators designed according to cultivation or research requirements. Stock nutrients solutions are prepared, and pH is adjusted prior to being used in tanks. Pressurized pumps are controlled by timers in a set of intervals frequency to mix and spray the nutrients solution to atomizers that provide a fine misting to the root zone to sustain hyper-growth.

Other pumps might be used to maintain nutrients cycle and flow within the closed system. Other designs include sensors to monitor the nutrient's level, temperature, humidity, EC and pH. Those apparatuses provide real-time tracking of environmental variables that might affect plant growth and developments. While the belowground parts of the plant are grown in rich nutrients spray, the upper parts (leaves, stems, and crown) are grown above the wet zone (Lakhiar et al 2018).

Along with the continuous supply of nutrients directly to the root system, the air culture prevents mechanical injury usually associated with soil's particles and properties. The interval frequency of the misting spray provides a measurable nutrients concentration uptake within the plant during the growing stages. Another advantage of the adjustable interval frequency that can accommodate varying growth stages dynamic and requirements.

### 12.2 Perennial & Fruit tree in aeroponics:

A study by Peterson & Krueger reported that perennial plants were maintained for 13 months in aeroponics system (Peterson and Krueger, 1988). The first successful report of apple grown in a misting environment was described by Vyvyan and Travell in 1953. However, in 1944 Koltz was the first to investigate the citrus and avocado root's disease by implementing the first vapor misted methods (Peterson et al., 1991).

Tamarix aphylla (L.) Karst. is halophyte tree from an agroforestry species had been tested in aeroponics to explore the potential of aeroponics system for clonal propagation and to compare conventional propagation methods to aeroponics in response to different concentration of various root promoting auxins on adventitious rooting (Sharma et al., 2018). They found a significantly higher number of roots and root length when grown in aeroponics rooted stem cuttings as compared to stem cuttings rooted in conventional soil propagation system. Mehandru *et al*, 2014 found that aeroponics can be successfully utilized to easily obtain root biomass of medicinally important plants like *Caralluma edulis, Leptadenia reticulata*, and *Tylophora indica* compared to difficult and expensive conventional soil growing method.

Significant growth and performance in terms of yield and quality were observed in strawberry growing in the aeroponics system. A study found that the maximum leaf area, largest crown diameter, highest shoot fresh weight and root fresh weight, highest shoot dry weight, and root dry weight was observed in aeroponics open-trough grew strawberry plants compared to conventional plantation (Karimi *et al*. 2013). They also reported that earliness in flowering and highest TSS was recorded in these plants as well as the highest number of flowers and fruits and total fruit weight per plant along with

higher marketable fruits.

### 12.3 Minirhizotron

Another reason why root's behavior is not fully understood for being buried under the soil which physically obstructs monitoring growth development with time.

There is no single recommended technique to study root development and architecture in soil. Several methods have been investigated in literature (Majdi et al., 2005). Those methods can be grouped into destructive and nondestructive methods. The destructive methods require collecting soil cores, the use of in-growth bags (van Noordwijk, 1993), and analysis on fine roots (Gaudinski et al., 2001; Matamala et al., 2003) trenching, , anatomical root drawings, and pinboards (Rewald and Ephrath 2013; McMichael and Zak 2006). Although, some of those techniques are still being used due to their destructive nature provide less information to track roots changes in response to their rhizosphere.

Nondestructive methods implement the use of minirhizotrons (Hendrick and Pregitzer, 1996; Johnson et al., 2001) and ground-penetrating radars (Stover et al., 2007). Rhizotrons have been used since early as 1900 by implementing underground-glass walled as observation windows (Busch et al. 2006). This method allows scientists to monitor the root's development and their interaction with soil and the rhizosphere microorganisms in situ over seasons. In 1937, Bates presented what he proposed as the first miniature rhizotron when he published his article "A Device for the Observation of Root Growth in the Soil" (Bates 1937).

Rapid root growth and expansion require more frequent observations and repeated monitoring of the root developments. Hence, some minirhizotron techniques implement

a camera to document those growth changes and facilitate measurements and assessments. This also allows frequent calculation of fine-root length production and mortality and turnover (Majdi 1996).

## 12.4 Components & Mechanism.

### Observation Tubes

Minirhizotrons use transparent observation tubes that are inserted in the soil. Several materials have been reported to be used in minirhizotron; polycarbonate (van Noordwijk et al. 1985; Box and Johnson 1987), polymethyl 2-methyl propenoate (or polymethyl methacrylate [PMMA], known as acrylic, Perspex, Plexiglas, or Acrylite; Itoh 1985; Vos and Groenwold 1987; Kloeppel and Gower 1995), and cellulose acetate butyrate (CAB or butyrate; Box et al. 1989; Hendrick and Pregitzer 1992; Wells and Eissenstat 2001; Yang et al. 2003) have been used. Selection of tube's materials depends on; experimental setup, image resolutions and the experiment's duration. However, Withington et al. (2003) found that the type of material of the tube may causes affect the root production and phenology of apple roots. They found a higher root production and roots turned light brown later and lived longer around glass tubes when compared to roots production growing near acrylic and CAB tubes. Moreover, roots grown next to CAB tubes turned brown faster than next to acrylic tubes. They reported that the survival of root was also shorter near CAB tubes in three of four deciduous hardwood species but shorter near acrylic tubes for three conifer species. (Rewald and Ephrath, 2013)

Generally, a rigid tube with less affected by soil minerals and compaction is essential to maintain durability and image quality over the experiment time.

Tube inner diameters ranged from 13mm (Boroscope; Upchurch and Ritchie 1983)

to 64mm (van Noordwijk et al. 1985). When roots intersect with those tubes, it allows viewing the root by a miniature still or video-cameras. Those tubes have been installed in a different way, vertically (90°), horizontally or at an angle (Johnson et al., 2001). Minirhizotron tubes set up depends on the study's requirements and the soil types. Estimation of root growth and direction should be considered when selecting the insertion angle to enable proper positioning of tubes. Soil should be equally distributed around tubes during the setup to reduce air gap and funneling effect (Rewald and Ephrath 2013). Tubes should be labeled and if multiple observation windows are required per tube, then each observation window needs to be labeled as well. Tubes also need to be sealed at the end to prevent moisture accumulation, light and thermal fluctuation inside it (Levan, Ycas, and Hummel 1987). When using observation tubes in the field, special consideration must be taken into account to ensure the proper installation of the tube. The study location's soil should be analyzed for bulk soil density and the required depth prior to the setup. This to ensure the site is free from big stone or other objects that can interfere with the tube or impede the insertion of tubes or requires auger for installation (Smucker, 1990).

**Rhizobox**

Rhizobox has been used when soil homogeneity is a factor that might influence root development. When monitoring root growth using rehizobox, soil structure and properties should be analyzed prior to the setup. Soil physical and chemical properties have a direct impact on root distributions and water infiltration to the rhizosphere. The soil has to be maintained moist if it known to forms cracks when drying (Dubach and Russelle 1995). The soil used in rhizobox should be settled and have a uniform

distribution around the observation tube to prevent the formation of the gap. Voids formed by gaps due to soil shrinking and compaction will influence or reduce root distribution. (Johnson et al., 2001). Small spaces between the outer surface of the OT and the soil will create a low-resistance path that can positively influence the root growth, branching, and survival (van Noordwijk et al., 1985; Volkmar, 1993). These voids spaces will accumulate moisture to condense on the outer surface of the tube that obstructs the root observation. (Rewald and Ephrath, 2013).

**Image Capturing Devices**

Cameras are also varying in their specification and outputs. Since the early uses of minirhizotron methods, several types of camera and images instruments have been used. Fiber optics, endoscopes, borescopes, root periscopes, and telescopes have been used and developed to improve image quality (Johnson et al. 2001). Although, it is necessary that cameras are compatible with the dimensions of the tube. Other image devices features include the production of high-resolution images for further image analysis. Since tubes are inserted in the soil, cameras should be able to take images in dark condition while maintaining distinguishable background from the fine root. The camera also needs a portable power supply for field operation with zooming capacity to study the root details (Allen et al. 2007).

**CI-602 Narrow Gauge Root Imager.**

Recently, digital cameras are being used to conduct faster and allowing more comprehensive image processing. Others are fitted with motorized mechanisms to facilitate intervals image-taking and reduce manual operations. Others are designed to enable 360° image taking while scanning the minirhizotron's tube like the CI-602 a narrow gauge in situ root images from CID Bio-Science (CID Bio-Science, Inc, WA,

USA). The same root imager is being used for this study which is the only commercial scanner designed especially for minirhizotron systems. This root imager developed with a modified charge-coupled device (CCD) where a flatbed scanner is used to capture root images. The CCD-type scanners are chosen due to their larger field depth (Dannoura et al. 2008). The CID- root scanner can take a 360° image of the soil–tube edge, by capturing the tube soil profile producing a picture size of 20 cm wide × 22 cm high. By these dimensions of an observation coverage, it reduces the time of taken multiple images per frame and thus cost-effective in labor and measurements analysis. It facilitates correct image processing and thus a better data interpretation due to continuous parts and branching of the root system can be observed, captured and studied. (Rewald and Ephrath, 2013).

The CI-602 can produce a high image resolution up to 1200 dpi which considered a reliable for detecting small changes in root behavior and architecture. This resolution also allows post–image digital zooming on picture details. However, using the higher resolution required longer scanning time, thus usually lower resolutions between 300–600 dpi were found adequate. The scanner is equipped with a standardized lighting and automatic focusing to facilitate root imaging under dark soil profile.

**Image processing software**

Earlier images processing was done manually by tracing root on the transparent sheet (Cheng et al. 1991). Nowadays, many specialized computer software are used to process images produced by root scanners and automatically track root development and changes. Several computer software are used to analyze minirhizotron images, such as Rootfly (Birchfield and Wells 2006), RooTracker (Duke University, Durham, NC),

Root Measurement System (Ingram and Leers 2001), and WinRHIZO Tron (Régent Instruments, Quebec, Canada). The common user's interference with image processing requires a manual root tracing. This includes tracing roots on the image in a computer screen along with the roots and setting root's diameters (Rewald and Ephrath 2013). Another interactive software designed to analysis root images called 'ROOTS' which was programmed by Hendrick and Pregitzer (1992) that can detect root changes over time by identifying the same roots on current observation and compared it with previous images. Very recently, a root images software called RootSnap® was introduced which allows the user to trace roots interactively using a touch screen input to facilitate accurately and easily tracing of the root system from minirhizotron images. It quickly measures root length, area, volume, diameter & branching angle (CID Bio-Science Inc., WA).

RootSnap® software can center the root automatically by implementing tracing enhancements "Snap-to-Root" which snaps root tracing points. This software also allows users to optimize scanned images for more accurate processing by integrated image enhancement features and thus eliminates the need to use additional image enhancement software. The observation images can be used to monitor root growth, dynamics and behavior over time and root disease along with microbial activities near the rhizosphere. It uses familiar commands to organize and store images and files in a common open file format (XML) and supports exporting data to other software for further analysis.

### 12.5 Significances of non-destructive root monitoring techniques.

Proper observation of a tree's root system in a non-destructive method is a key for

accurate monitoring and assessment of the root development. Thus, special techniques are required to study spatial distribution, turnover, and root's growth. Conventionally, destructive samplings methods are used to evaluate the root spatial distribution and growth by soil coring, in-growth cores, whole root-system excavation, and trenching (Johnson et al., 2001; Wu et al., 2005). Other techniques are used to make repetitive observations of the root development in a non-destructive way which includes rhizotron and minirhizotron (Johnson et al., 2001). These methods provide a unique opportunity to track new root emergence and turnover to evaluate the dynamic changes of root developments (Majdi and Kangas, 1997). However, still, root's development is not well studied under stress rhizosphere environment.

The successful adaptation of soilless cultures in commercial-scale opens many opportunities to implement these techniques in research aiming for a better understanding of root's development and architecture. Aeroponic is being considered as a common advantage for both growers and plants researchers. It provides a highly profitable product for users and a closed growing system for scientists where all factors affecting plant's growth and developments are controlled. It also allows optimizing those factors to influence and speed up the growth and in returns reducing time and labor cost Nir, I. (1982). Whereas the most valuable contribution on the aeroponics to scientific research is the ability for repeated and replicated evaluations of the root's development in nondestructive methodology. Moreover, assessing the root characteristics and architecture in response to changes in nutrient's pH or root's environment condition such as humidity and temperature. It is also allowing real-time monitoring of the root system behavior within when the system is equipped with a time-

lapse camera.

The unique advantages of aeroponics to study root architecture is that root's development undergoes no resistance or unfavorable conditions to expand. The effect of tropism gravitropism is not affected by soil particles, moisture or nutrients availability. Thus, the true phenotypic plant's characteristic will be strongly obvious. The direction of the root's movements is not affected by an external force. Here the root's distribution is clear and detectable in non-destructive methods.

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# CHAPTER TWO

## Growth and Fruiting Performance of Honeycrisp™ Apple Trees Grafted on 8 Rootstocks in Response to Soil pH.

*Abstract*

Apple (*Malus* × *domestica* Borkh.) trees are not always grown in a favorable orchard condition. One of the soil properties that can influence apple tree growth and fruit quality is the soil pH due to its effects on nutrients uptake leading to adverse effects on the tree's performance. However, in many cases, the implementation of new apple rootstocks represents more sustainable solutions to alleviate site challenges and tolerating unfavorable growing conditions.

In this study, eight apple rootstocks (G.11, G.41, G.935, G.202, G.214, M.9T337, B.9, and M.26 EMLA.26). Honeycrisp™ scion cultivar was grafted on all rootstocks and planted in 15 gallons pots-in-pots with growing medium pH adjusted to 5.0, 6.5 and 8.0. at Cornell orchards, Ithaca NY. Rootstocks performances were evaluated in terms of tree growth, nutrient status in response to three pH treatments (Low: 5.0, Medium: 6.5, and High: 8.0) for two growing seasons from 2017 to 2018. Fruit yield and quality were also evaluated among rootstocks from one fruiting season.

TCSA increase shows a highly significant difference at $P \leq 0.001$ between rootstocks with a maximum increase reported in rootstock G.935 and the lowest TCSA increase was found in B.9. However, no significant difference was found in the effect of the pH treatments in TCSA.

Statistical analysis showed that soil pH treatment affects fruit's peel nutrients and showed highly significant difference at $P \leq 0.001$ on P, Ca, Mg, and Fe. and a significant difference at $P \leq 0.05$ on S, B, and Zn.

The leaf nutrients analysis showed higher values of K, Ca, Mg, S Fe and Mn at low pH within pH treatments. However, higher P and Zn were found at high pH. However, no significant difference was found in total soluble solids %, fruit's firmness, number of fruit, bitter-pit incident percentage. The highest fruit per tree was found on rootstock G.41 under pH experiment. And the lowest bitter pit % was reported in G.935.

Rootstock G.11 showed a bigger fruit size, weight, and length while G.935 showed better red skin color percentage among rootstocks. All fruit maturity parameters except for yellow and red skin showed a significant difference in all soil pH treatments. However better values of fruit's weight, size, and length were found at high pH within pH treatments.

## 1. Introduction

Planting and maintaining an apple orchard (*Malus* × *domestica* Borkh.) requires about 20-years of obligation. Proper site selection has been an important factor in successful and sustainable apple orchards. Geographic location, climate, topography, and soil condition are the most critical factors that determine growers' decision in selecting orchard sites. However, site selection is not always optimum in terms of soil chemical composition and properties. Besides cultivar selection, other key decisions made by growers include rootstock selection, irrigation, pests and disease management, and fertilization which also affect orchard viability and profitability. During the life of the orchard, growers can change some of the above-mentioned management choices, however, once a rootstock is in the ground, it cannot be changed without ripping out the whole orchard. Thus, apple growers are in search of a rootstock with optimum field performance various soil and climate conditions and that can positively interact with the scion cultivar for maximum economic productivity.

Apple trees are not always grown in optimal growing conditions and many biotic and abiotic stresses may hinder the production in many growing regions. These challenges will consequently reduce trees growth, yield and decrease fruit quality.

Rootstocks are widely used to control tree vigor and enhance precocity of apple scion varieties. They also influence tree nutrient status, yield efficiency, fruit quality and other aspects of tree performance. Molecular studies of apple genomics show that rootstocks can impact the performance of the scion differently (Fazio et al., 2012a(Jensen et al., 2014, 2012). Adopting rootstocks with more tolerance to various stresses represents the most economical long-term solution to abiotic and biotic stress problems encountered

in the field.

To increase apple production in many countries around the world, various promising apple cultivars have been introduced to new apple growing area around the world that show a potential adaptation to different ecologies. However, prior to their introduction, evaluations need to be carried out on different apple rootstocks to determine their adaptability and productivity of specific variety/rootstock combinations in particular geographical region.

A specific current case is the introduction of the variety Honeycrisp which is weak growing and likely needs different rootstocks than traditional varieties. Although Honeycrisp™ is not considered a new apple variety, it is still gaining remarkable popularity among apple growers and consumers. Its exceptional balance of crispiness, texture, juiciness, balanced flavor and aroma have been favored by consumers (Zhang et al., 2010). It was predicted by the US Apple Association to be in fifth place for America's favorite apple by 2020 and is expected to be the third-most-grown cultivar (Bloomberg, 2019). However, Honeycrisp requires specific horticultural and post-harvest practices to maintain sustainable and profitable yields. Eventually, this has led to laborious flower and fruit thinning to prevent small size and poor quality fruits in heavy bloom seasons (Forshey, 1986). Additionally, Honeycrisp fruit is prone to bitter pit, a physiological disorder that has long been associated with low fruit Ca content (Schupp et al., 2005, 2001), localized Ca deficiency in fruit, (Cheng and Sazo, 2018; Schupp et al., 2005, 2001, 2001, Fazio et al., 2018a). This disorder could be also associated with soil's pH which determines nutrients availability within certain pH ranges. Honeycrisp also experiences fruit coloring problems, appearance defects, and susceptibility to leaf disorder referred to

as zonal chlorosis (Chen and Cheng, 2010, 2004). Along with bitter pit, the fruit is prone to scald, soft scald, and a tendency to ferment due to skin permeability problems (Rosenberger et al., 2001)

### 1.1 Purpose and Significance of the Study.

Many concepts have been proposed to improve 'Honeycrisp' yields and fruit quality. It has been well documented that apple rootstocks influence tree vigor, enhance precocity (Marini and Fazio, 2017), influence transpiration and hydraulic resistance (Adams et al., 2018), modulate mineral nutrition, (Andziak and Tomala, 2004), and affect fruit ripening (Autio et al., 1996) of apple scion varieties. Apple root system shows different growth configurations that influence their water and nutrients uptake efficiencies related to their genotypic background (Fazio et al., 2015). By selecting rootstocks with more tolerance to various stresses, economical, long-term solutions to abiotic and biotic stress problems encountered in the field can be provided at planting.

Soil type and soil pH are two key soil properties that impact how rootstocks influence nutrient uptake of the scion cultivar. However, rootstocks vary in their response to the effect of these soil factors. The evaluation of the available commercial rootstock's performance in unfavorable growing conditions using modern screening techniques could improve apple production. Although extensive research has been carried out evaluating the effect of apple rootstocks on many commercial scion cultivars, no single study exists evaluated the performance of Honeycrisp scion performance on Geneva® rootstocks under various soil pH.

This Ph.D. study focused on the interaction between apple rootstocks and abiotic soil conditions, specifically soil pH. The study covers tree growth performance, fruit

quality, leaf and fruit nutrients composition of ‘Honeycrisp’ grafted on eight widely used apple rootstocks. These evaluation studies provide knowledge-based recommendations for rootstock selection and ultimately increasing orchard productivity which can bring mutual benefit for both, the grower and the consumer.

### 1.2 Theoretical Basis for this Study

This experiment was designed to evaluate eight apple rootstocks in response to three levels of soil pH to try to answer questions regarding the growth performance and fruit quality of ‘Honeycrisp’ apple. We compared five new Geneva® apple rootstocks (G.11, G.41, G.935, G.202, and G.214) with three widely used apple rootstocks (M.9T337, B.9, and M.26 EMLA.26). The scion variety Honeycrisp variety was chosen as the target scion for evaluating fruiting and fruits quality due to the many production issues of this popular new variety.

To assess the effects of soil pH, we measured trees growth parameters (tree height and trunk cross-section area) in the first and the second growing season (Russo et al., 2007a). However, fruiting was only allowed in the second growing season and thus fruit quality was assessed in that season. Fruit quality descriptors were collected at harvest (yield, skin color, fruit size, and weight) and after three months in storage (total soluble sugar, firmness, fruit peel nutrient concentrations, and physiological disorders).

### 1.3 Justification and hypothesis.

There is limited information about the response of ‘Honeycrisp’ fruit quality when grafted onto Geneva apple rootstocks grown in different soil pH. Also, Geneva rootstocks have not yet been fully characterized with regard to their interaction with soil pH. This research hypothesized that while soil pH influences soil nutrients availability, each

rootstock would react differently, resulting in differential nutrient uptake which in turn would affect Honeycrisp growth and fruit quality.

Thus, the objectives of this study are:

1. To evaluate the performance of 8 rootstocks in response to different soil pH levels in terms of growth performance and fruit quality.
2. Compare the performance of several new Geneva apple rootstocks (G.11, G.202, G.935, G.41, and G.214) with traditional widely used rootstocks (M.9-337, EMLA. 26 and B.9).
3. Assess nutrient uptake capacity of each rootstock at different soil pH levels.
4. Accurately determine the optimum soil pH for each apple rootstock.
5. Evaluate Honeycrisp™ cultivar growth under a range of soil pH in terms of tree growth and fruiting.
6. Assess the fruit quality and bitter pit incidence and correlate with soil pH.

Results from these evaluations will assist in breeding apple rootstocks to accommodate diverse soil condition particularly soil pH..

## 2 Materials and Methods

### 2.1 Plant Materials

**Budwood collection:**

Honeycrisp™ scion wood was collected from fields Hansen 12 rows (1,5,9,13 and 9 trees from 17), RS02 NC-140 rows (1,2 &3), Hansen 14E, Hansen 15 rows (7,6,5 & 4) from Cornell AgriTech, Geneva, NY. Scion-wood were collected twice on 3/24/2015 and 4/2/2015 and stored in cold storage in wooden crates covered with sawdust and moistened frequently until ready for grafting. All scion woods were one-year-old wood with a minimum of 12 buds per scion. Rootstocks for this experiment were supplied from Willow Drive Nursery, Inc., Washington and a from Dr. Fazio Geneva apple rootstock breeding program (Table 2). The initial proposal was to evaluate 15 apple rootstocks but due to the complexity and unavailable field area, it was then downscaled to 8 rootstocks (Table 3.).

Table 2.Apple rootstocks used in the project experiment.

| Rootstock | Diameter | Source |
|---|---|---|
| CG.5087 | < 1/4" | Dr. Fazio program |
| CG.5257 | < 1/4" | Dr. Fazio program |
| G.841 | < 1/4" | Dr. Fazio program |
| CG.4292 | < 1/4" | Dr. Fazio program |
| M.9-337 | 3/8" | Willow Drive Nursery |
| EMLA. 26 | 3/8" | Willow Drive Nursery |
| G.202 | 3/8" | Willow Drive Nursery |
| G.11 | 3/8" | Willow Drive Nursery |
| G. 969 | 1/4" | Willow Drive Nursery |
| G.210 | 1/4" | Willow Drive Nursery |
| G.41 | 3/8" | Willow Drive Nursery |
| G.935 | 3/8" | Willow Drive Nursery |
| G.214 | 1/4" | Willow Drive Nursery |
| B.9 | 3/8" | Willow Drive Nursery |
| G.890 | 3/8" | Willow Drive Nursery |

**Grafting rootstocks by Honeycrisp™ scion.**

Honeycrisp™ scion cultivar was bench grafted in spring 2015 on all rootstocks and then kept in cold storage in plastics bins covered with sawdust and moist frequently until ready for transplanting. Prior to transplantation, trees were placed outside in a shaded area for acclamation for three, four and five hours a day respectively.

**Transplanting**

Trees were transplanted into a liner bed in nursery at Cornell AgriTech, Geneva, NY in early summer May 29th, 2015 for 18 months for establishment. Trees were irrigated and fertigated frequently by NPK and with additional applications of Osmocote Pro with Micronutrients 19-5-9 (ICL Fertilizers, USA) as required. After scion started to grow, only a single leader was allowed to grow and splitting and removal of all side shoots was done frequently. Regular orchards managements practice such as weeding and pest management was carried out during the establishmentt stage in the nursery. Immediately after planting, bamboo sticks were placed next to the plants and tied to the stick at several levels to support the tree's growth. This was necessary to get a straight stem tree.

Table 3.Apple rootstocks evaluated and their descriptions.

| Rootstock | Type | Parentage | Tree size | Origin |
|---|---|---|---|---|
| **B.9** | Dwarf | M.8 x Red Standard | M.9 | Michurinsk State Agrarian, Russia |
| **M.9T337** | Dwarf | *Malus pumila* Mill. var. *paradisiaca* (L.) | M.9 | East Malling (UK) |
| **M.26EMLA** | Dwarf | M.16 x M.9 | M.26 | HRI-East Malling (UK) |
| **G.11** | Dwarf | M.26 x Robusta 5 | M.9 | Cornell University-USDA (USA |
| **G.214** | Dwarf | Ottawa 3x Robusta 5 | M.9 | Cornell University-USDA (USA |
| **G.202** | Semi-dwarf | M27 × Robusta 5 | M.26 | Cornell University-USDA (USA |
| **G.935** | Dwarf | Ottawa 3 × Robusta 5 | M.26 to M.7 | Cornell University-USDA (USA |
| **G41** | Dwarf | M.27xRobusta 5 | M.9 | Cornell University-USDA (USA |

**Promoting feathers and lateral branching**

In the early spring of 2016, when the scion trunk circumference diameter was at least 10 mm, the main shoot was headed back in knip-boom cut to promote scion growth.

The cut was made at a 70cm height above the soil line. All shoots and suckers below grafting union were removed continuously during the growing season. Three applications of 500ppm MaxCel (Valent U.S.A. LLC) by knapsack sprayer were used to promote feathers and lateral branching. Maxcel is a benzyladenine contains cytokinin, a synthesized plant hormone, that involved in regulating cell division. It has been used as a chemical thinning agent to adjusts crop load and to stimulates cell division to improve fruit size (Sazo and Robinson, 2012; Szot et al., 2018). Combining the use of Maxcel and the knip-boom cut was also suggested for successful production of well-feathered tree in the nursery (Sazo and Robinson, 2011). This was performed to shorten the nursery cycle and to produce a two-year-old tree that can bear early fruiting (Bielicki et al., 2002).

**Up-rooting nursery trees and pre-planting tree's screening.**

Trees were uprooted in the fall of 2016 using a flat spade to uproot all trees with maximum roots. Trees were then cleaned from the soil and grouped into rootstocks and kept in cold store till spring 2017. During the spring of 2017, trees were screened by selecting uniform trees from each rootstock, re-labeling and 8 rootstocks were prepared for transplanting (Table 3).

**2.2 Soil testing**

In 2015, several topsoil samples from different suppliers were tested for soil pH and total nutrients analysis. Series of long-term buffering capacity testing was conducted in order to aid in selecting appropriate base soil for this experiment. The soil test experiment lasts for one year from 2015-2016 in-vitro in soil laboratory to find the appropriate combination rate of top screen soil, perlite and the amount of pH adjuster elements (Calcium or Sulfur) in order to test for pH stability and nutrients availability. Soil samples were also tested to check the malleability to adjust its original soil pH and

the buffering capacity using a various range of combinations ratio.

Top screened soil from local soil supplier (Cayuga Compost P&S Excavating LLC, Trumansburg, NY) which was previously tested for pH buffering capacity and nutrient availability was found to be suitable for the purpose of this experiment. The soil sample was then further screened in a 1.27cm mesh screen and sent for nutrient analysis. The soil was then tested for pH buffering capacity by adjusting the pH to acidic and alkaline by addition of elemental sulfur or calcium carbonate respectively. Samples were then monitored for one year in lab and soil pH was evaluated and observed. The desired soil's pH range was achieved by optimizing the correct mix combination of soil: perlite: sulfur/calcium carbonate.

**Soil preparation and pH adjustment**

The original soil's pH of the selected soil was 7.3 and was adjusted to 5.0, 6.5 and 8.0. +/- 0.3. Perlite was added to the soil mixes on ratio 1:4 to improve soil structure and help in soil aeration when the mixture was used in pots. A concrete mixer was used to prepare the soil mixes to maintain a homogenous mix. Mixed with perlite in 1:4 ratio and elemental sulfur (99.9% sulfur Duda Energy LLC, Decatur, AL) were added to lower pH (Acidic) or calcium carbonate (CalCarb AC3, Mississippi lime) to raise the pH (alkaline). The treatment soil medium's pH was adjusted to 5.0, 6.5 and 8.0 and was filled in 55 liters plastic pots blow molded - grip-lip pots (top diameter 44. cm & 43cm height).

**Raising soil pH.**

Fine lime powder of ground calcium carbonate $CaCo_3$ (CaLCarb AC3. Mississippi lime, Geneview, MO) was used to raise and adjust the soil pH. A sequence of experiments was conducted on the selected soil to determine the appropriate rate. In order to select the correct application rate, a dose-response curve was generated from many soil mixes.

When the correct dose was known, it was mixed using the concrete mixture to ensure homogenous soil uniformity.

**Lowering pH.**

Elemental sulfur (S) was used to lower soil pH. The soil texture, amount of soil organic matter, present pH, and the desired pH were all assessed to determine the amount of elemental sulfur needed. For more rapid results in lowering pH, elemental powder sulfur (99.9% sulfur Duda Energy LLC, Decatur, AL) was used to lower the soil pH by mixing in the concrete mixture.

**2.3 Site Preparation**

An experimental site was assigned in the Cornell Orchards and was plotted in 7 rows with 3.35 meter between rows by 1 meter within a row. Each row was excavated using a mini-excavator to make trenches (90 cm wide X 90 cm deep) along the row. Trenches were used to lower the impact of cold winter damage and to reduce temperature build up in pots during the summer. Trench's bottom was leveled manually, and drainage lines were lined and covered with gravel stone.

About 2 inches layer of stone gravel was placed at the Pot's bottom and then topped with soil that was previously pH adjusted. Each filled pot was stacked into another identical pot to form pot-in pot scheme. Pots were assigned a location within the rows according to the spatially balanced complete block designs to eliminate the effect of field variability. (Spatially balanced Design). The experimental field was equipped with a manual dosatron fertilizers injectors system (SuperDos® 30, Dosmatic USA, TX, USA) to supply regular fertilizers through irrigation lines.

**2.4 Transplantation into the pots**

On spring 2017, tree were screen and selected based on even hight and number of side branches (Table 4). The roots of the selected trees were cleaned from the soil and

washed with water and then transplanted into designated pots by holding the tree in straight upright and placing the root crown in the center of the pot while maintaining the grafting union to be 3 inches above the soil line. The same adjusted soil was used to backfill pots and was packed and toped to allow the soil to settle after irrigation. Trees were planted into pots while pots on the designated spot into the trenches to avoid moving trees after planting and because pots were so heavy to transfer after planting.

The next few weeks, wire trellis were then fixed, and the irrigation system was installed and backfilling of trenches was completed. Trees were irrigated twice a week from June till September. All trees were fertigated with Hoagland's solution throughout the growing season by supplying 150ppm N twice a week.

**2.5 Experimental design**

Each pH treatment was replicated 5 times with 3 trees per replicate in a spatially balanced complete block experimental design. Eight apple rootstocks were evaluated in this experiment by comparing 5 rootstocks from Geneva series (G.11, G.214, G.202, G.935, G41) and compared by 3 widely used rootstocks (B.9, M.9T337, M.26EMLA). Each rootstock were replicated five times using three trees per replicates under three range of soil pH (5.0,6.5 and 8.0). Trees were planted in seven rows that each measures 30m long to accommodate 360 trees in this experiment. Few trees were used as untreated control with unadjusted soil pH that was at pH 7.3.

**2.6 Base nutrients partitioning analysis**

A set of five trees from each rootstock were selected for total harvest nutrient analysis before transplanting into pots. Those trees were not grown in the pH experiment and resembled samples from each rootstock in terms of average height, trunk cross-section area (TCSA) and a number of branches. Trees were washed thoroughly to ensure clean

from soil debris or chemical spray. Each tree was then partitioned by cutting to; branches, trunk, upper shank, lower shank, and roots. Tree's parts (organ) were cut into small sections and then dried in a forced-air oven at 70℃ for two weeks. The dry weight of each part was recorded at the end of drying procedures. Each sample was ground to pass a 3mm mesh screen and measured for total elemental analysis using wet combustion analysis and inductively coupled plasma emission spectrometry (ICP).

Samples were digested with nitric and perchloric acids using the Vulcan 84 automated digestion system. (Questron Technologies Cor. Mississauga Ontario Canada). About 0.30 to 1.0 grams of sample were weighed into 50ml Teflon containers plus 0.25 ml of 80 ug per ml of yttrium. This is used as an internal standard. The digestion system automatically using syringe pumps added 5.0 ml of 67-70% Omni Trace nitric acid plus 3.0 ml of environmental grade 70% perchloric acid from GFS chemicals Columbus, Ohio. The samples are heated to 110℃ over 40 minutes and held for 60 minutes. The temperature is increased to 160℃ over 20 minutes and held for 15 minutes. An additional 1.0 ml of nitric acid is added, and the samples heated an additional 20 minutes at 160℃. After cooling 20.0 ml of 18meg water is added. The solutions are then analyzed using an axial viewed ICP-OES. (Spectro Arcos FHE12 made in Kleve Germany).

All the results were verified for accuracy by inspecting the spectral display for each element reported for all the samples. This was determined by multiplying the instrument detection limits by the dilution factor which is about 67 for a 0.3g sample. In each organ, the nutrients were calculated based on their concentration and dry weight data. The base nutrients analysis was used to emphasis on sampling plant parts that, when the nutrient concentration is compared and correlated. Total carbon and nitrogen were tested using C

and N by an element's analyzer. All nutrients analyses were conducted at Cornell Nutrients Analysis Laboratory, NY, USA

**2.7 Soil testing and pH monitoring.**

After two weeks from transplanting and irrigation, a soil core was used to collect soil samples from a depth of about 20cm in pots to test for soil nutrients availability and pH. Another sampling was repeated again in the fall of 2017 and fall 2018. Soil's pH was monitored monthly by taking soil samples and pH testing to check for pH stability. Soil's pH was tested using portable pH meters (Hanna Instruments, HI 9813-6 Waterproof PH EC TDS Temp Meter.) by taking samples from 10 same pH pots and were mixed together and then subsamples 1 gram from the soil mix and dissolved in 10 mL DI water and let it settle for an hour and then taking the reading. According to the pH reading, additional sulfur was added to lower soil pH in the pots or calcium to raise the soil pH.

**2.8 Field horticultural practices**

At planting, broken and damaged branches were pruned while those trees with many feathers (more than 15 lateral branches) at planting were minimally pruned to provide a balance between the scions and root to encourage growth.

In the 1st leaf, all flowers were manually removed to help trees to establish growth in the pots. The next growing season, all flowers on the upper 1 meter of the leader were removed and lower flowers were allowed to set and manually thinned by keeping only two flowers per cluster by setting 1.5 flower buds for each fruit to achieve the target of 4 fruits/ Trunk cross-section area. Further, when fruitlets were about 8-12mm diameter, manually thinned to one fruit per cluster.

Table 4.Average Initial tree's height and an average number of feathers at planting in 2017

| Rootstocks | Average height (cm) | Average number of feathers |
|---|---|---|
| B.9 | 130 | 16 |
| M.9T337 | 110 | 15 |
| M.26EMLA | 110 | 15 |
| G.11 | 130 | 21 |
| G.214 | 130 | 23 |
| G.202 | 130 | 25 |
| G.935 | 120 | 25 |
| G.41 | 130 | 19 |

Tree's training was planned to follow the tall spindle system by encouraging high feathering during nursery growth. The experimental plot was equipped with tree trellis on 50cm increment and trees were clamped to the vertical wire. The following spring (2018), lateral branches those larger than ½ the diameter of the leader and competing the leader's growth were removed and all smaller lateral branches were shortened by one-third. All trees were hand thinned to 4 fruits/$cm^2$ TCA in early June of the first year. Regular disease and pest management were maintained throughout the season. Trees were irrigated when required (every three days and daily during the hot days). Fertigation was also used weekly by applying 150ppm NPK plus 40ppm Iron.

**2.9 Data collection (Growth parameters)**

Tree growth parameters were recorded in terms of a tree's height and trunk circumference. Trunk circumferences were recorded at 20cm above the grafting union and the trunk cross-sectional area (TCSA,$cm^2$) was then calculated every spring and fall, (spring & fall 2017- spring & fall 2018- spring & fall 2019). Rootstock performance at each pH was individually assessed by comparing the mean growth difference between growing seasons (spring-fall) and between years in terms of tree growth (Height and TCSA), nutrient status, fruit yield, quality, and storability. Leaf nutrient levels, yield, and quality were assessed and evaluated among rootstocks from 2017 to 2019.

## 2.10 Leaf and soil sampling.

Early summer during July, ten mid position leaves on new extension growth were collected from three trees per replicates from 3 replicates for nutrients analysis. Samples were washed thrice with DI water and then oven dry at 70°C for 10 days. Samples were then ground and placed in paper bags. All nutrients analyses were conducted at Cornell Nutrient Analysis Laboratory using the same protocols mentioned earlier in base nutrients analysis in section 3.2.7.

## 2.11 Fruit's maturity and quality assessments.

Trees were harvested on September 25th, 2018 where each tree was harvested individually, and fruit was kept in separate bags and labeled with tree's id number, pH and replicates. Soon after harvest, fruits were assessed using the fruit sorting machine (Combi sort, GREEFA, GreenTech Netherland**)**. The sorting machine was set to measure fruit's weight, size, length and percentage of the color green, yellow and red by loading Honeycrisp assessment library.

Fruits were then placed in mesh bags and transferred to ventilated fruit bin and kept in a cold store at 3.3 degrees Celsius. After 5 months in cold storage, fruits were assisted for quality and maturity in February 2019. Maturity assessment was done by taking measurement of fruit firmness, total soluble sugar (TSS) and incidents of the better pit and soggy breakdown were calculated. Each fruit was spot peeled and firmness was measured from opposite sides at the point halfway between the stem and calyx end of the fruit. Fruit firmness was measured using a penetrometer fruit texture analyzer (GÜSS manufacturing (Pty) Ltd., South Africa). Total soluble sugar (TSS) was measured by subsampling of 10 edges (one edge from each fruit) and the juice was extracted and then tested by using refractometer (Hanna Instruments Inc, USA). Better bit incidents and

soggy breakdown were recorded for each tree.

### 2.12 Statistical analysis

Data from growth and fruit's parameters (Tree's height, tree TCS, number of fruit, firmness, TSS and physiological disorder incidents) were recorded and organized in "Microsoft Excel" (2016) before running the statistical analysis. Fruits parameters and dimensions were generated by the fruit sorting machine and parameters spreadsheets were generated by the machine.

The final analysis for the effect of each soil pH on tree performance and fruit quality were separately analyzed using analysis of variance with split plot design where rootstock was the main effect and the soil pH was the subplot. Each soil pH treatment has 5 replicates and 3 sub-replicates.

Mean separation of growth parameters (Tree height and TCSA), leaf nitrogen and carbon concentration, leaf nutrients, fruit quality and storage disorder, fruit maturity parameters, fruit peel nutrietns and fruit peel ratios were compared using Duncan's multiple range test with $P \leq 0.05$ and the appropriate errroro term for rootstock and soil pH and the interaction of the rootstock and soil pH. To determine the effect of soil pH, a regression analysis was used. A multivariate correlation trees growth and performance in response to between soil pH and rootstocks was also used to check for a factor correlation. Comparisons were analyzed between rootstocks, within soil pH range or nested interaction between both. All analyses were performed on SAS 9.4 statistical software (SAS Institute Inc. Cary, NC, USA).

## 3 Results

### 3.1 Soil nutrients analysis before pH adjustment:

The selected soil was tested in vitro for one year to estimate the pH buffering capacity (pHBC) and the titration curve was initiated to estimate the liming and sulfur requirements to adjust the treatment soil's pH. The total elements from the soil used in this experiment before adjusting the pH are noted in Table 5. The chemical composition was normal and was adequate to support the apple tree's growth. The original soil pH of this was near neutral (pH 7.37) and was found to be malleable to lower or raise to meet the experimental soil's pH. However, this soil has a medium (pHBC) that lead to stabilizing its original pH.

Table 5. Nutrients analysis of original topsoil before pH adjustment.

| Element | Value |
|---|---|
| Organic matter (%) | 5.94 |
| pH | 7.37 |
| Moisture (%) | 1.27 |
| Phosphorus (%) | 6.23 |
| Potassium (%) | 2.82 |
| Calcium (%) | 3.91 |
| Magnesium (%) | 3.38 |
| Sulfur (%) | 2.93 |
| Zinc (µg/g) | 10.8 |
| Manganese (µg/g) | 4.25 |
| Iron (µg/g) | 7.32 |
| Copper (µg/g) | 0.14 |
| Boron (µg/g) | 14.2 |
| Aluminum (µg/g) | 9.53 |
| Organic matter (%) | 5.94 |

### 3.2 Nursery trees performance

After transplanting trees to the nursery, trees developed a normal growth and established a uniform growth's height within rootstocks. Few trees died due to drying and biotic stress or unknown reason. Some grafted scions dried out however the rootstock continued growing so those trees were spring budded and kept as extra trees. Other sporadic trees from several rootstocks develop a zonal leaves chlorosis. This was a random symptom with no pattern associated with the rootstock or sol's pH. This symptom was explained by many studies reporting that excessive starch grain accumulation might be associated with imbalances in the source/sink relationships or abnormalities (Chen and Cheng, 2004; Zeeman et al., 1998). This may lead to inhibition of photosynthesis that may occur by sucrose accumulates which ultimately decrease the rate of photosynthesis (Eckardt, 2003). However, in Honeycrisp, the starch build-up is happening early in the season thus photosynthesis inhibition might not affect this cultivar during the fruit set and development (Snyder-Leiby and Wang, 2008).

During the first growing season, all rootstock produces a few numbers of feathers (lateral branches) thus, a knip- boom cut and MaxCel applications were suggested to improve the induction of lateral branches.

### 3.3 Effect of Knip-boom cut and Maxcel application.

Performing the knip-boom cut and the application of MaxCel promoted the production of lateral branches. A noticeable increase of side lateral branches was noted in all rootstock after performing the cut. Since knip cut was not a treatment in this experiment, no control untreated trees were used and thus, no records were taken with regard to a number of feathers developed in each rootstock, however, all trees develop between 12-

23 lateral branches. The satisfactory result obtained after doing the cut is in line with the result reported by Sadovski and Gorski who noticed a difference between knip-boom cut performed on apple trees when compared with budded trees (Sadovski and Gorski, 2003). Another study also supported the effect of knip boom cut to influence overall strength and promoting blind bud growth (Palmer et al., 2005). The successfully combined effect of the application of MaxCel with knip-boom cut matched many studies reporting increase feather on whips or poorly feathered trees (Sazo and Robinson, 2012, 2011; Szot et al., 2018).

**3.4 Base nutrients partitioning analysis**

The graph shows the mean distribution of nutrimental concentration over tree parts of each rootstock before grown in pH adjusted soil (Figure5). Nutrients analysis of total destructive harvest from 2017 shows the different allocation of nutrients within the tree's part. However, the nutrients show similar pattern within rootstock. Potassium (K) was higher at roots followed by branches in all rootstocks while Calcium (Ca) was in higher in branches and trunk. Other nutrients (Mg, P, and S) were following a similar pattern in all rootstocks (Figure 5). This was reported in many deciduous fruit trees (Porro et al., 2018; Tagliavini et al., 2000).

**3.5 Soil pH analysis.**

Lowering soil's pH was a slow process and the low (pH 5.0) treatment pots required a longer time to reach the required pH level which was reported by many research in lowering soil pH in blueberry fields (Hart et al., 2003; Haynes and Swift, 1986; Spiers and Braswell, 1992). It also needed additional applications of sulfur during the season. This because sulfuric acid needed to arise by microbial oxidation of elemental

sulfur and particles size, the smaller the particles of sulfur, the faster the reaction (Fliermans and Brock, 1972). In this study, lowering soil pH was not progressing as predicted and much additional sulfur powders were used to lower the pH to the treatment range. Manual incorporation of the sulfur was practiced in each pot during the first growing season. This led to damage some root and thus was decided to be mixed with water and applied it as a solution to avoid damaging the roots in the next growing season. Due to its nonpolar nature; sulfur was insoluble in water and thus a solution was prepared in the laboratory by dissolving in acetone under heat.

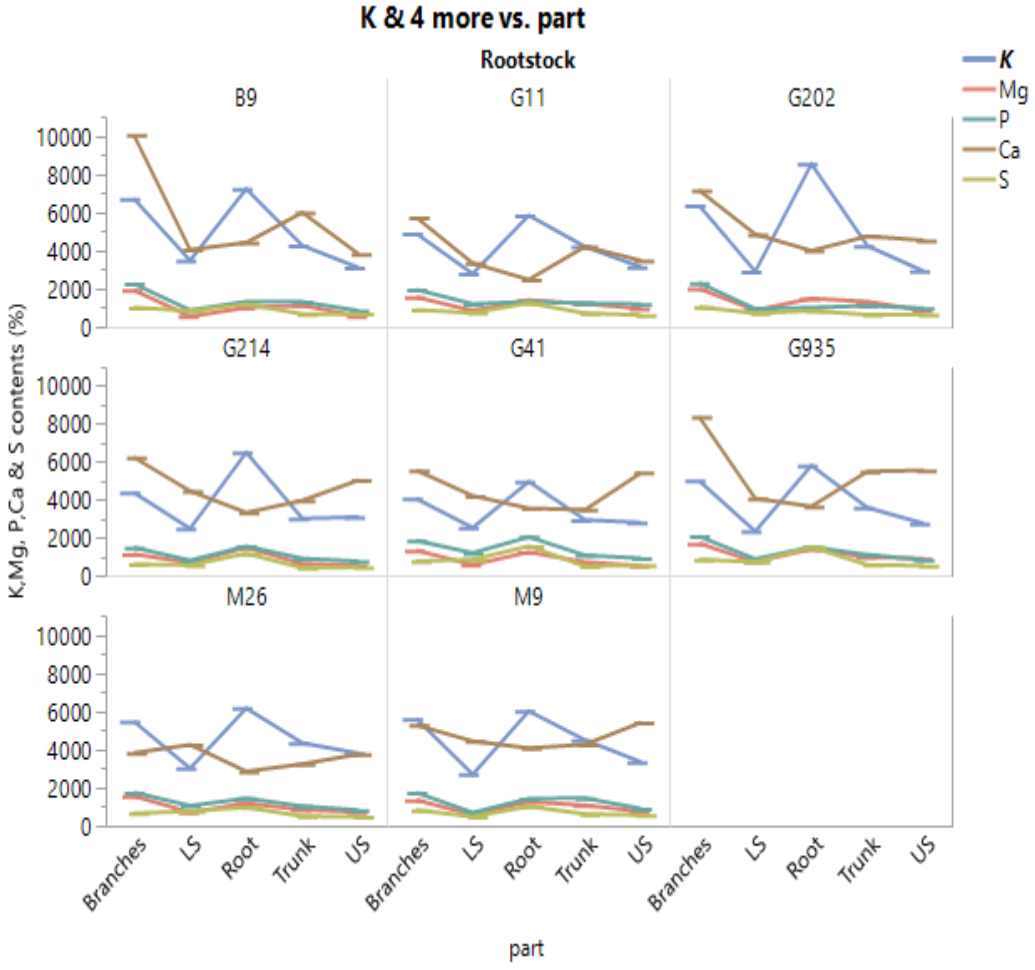


Figure 6. Nutrients concentration of rootstock parts from destructive total harvest 2017

It was added twice during the experiment to a few pots that showed pH lower than set pH by 1.5. Table 6 and 7 show the average soil nutrients analysis of samples taken from pots at each pH range at the end of the first and the second growing season in Fall 2017 and 2018. Thus the following range was adjusted in the analysing the results (low, medium and high).

Table 6.Soil's nutrients analysis after one growing season (fall 2017).

| Element | pH 5.0 | pH 6.5 | pH 8.0 |
|---|---|---|---|
| Organic matter (%) | 8.06 | 10.99 | 4.93 |
| pH | **4.27** | **6.71** | **7.94** |
| Moisture (%) | 1.45 | 1.42 | 1.35 |
| Phosphorus (%) | 3.33 | 5.29 | 3.23 |
| Potassium (%) | 12.45 | 29.38 | 14.12 |
| Calcium (%) | 3.04 | 3.93 | 3.76 |
| Magnesium (%) | 17.18 | 27.33 | 42.43 |
| Sulfur (%) | 5.02 | 3.85 | 4.15 |
| Zinc (ppm) | 2.08 | 2.15 | 1.21 |
| Manganese (ppm) | 0.83 | 0.18 | 0.15 |
| Iron (ppm) | 15.55 | 25.9 | 20.36 |
| Copper (ppm) | 0.43 | 0.33 | 0.26 |
| Boron (ppm) | 0.10 | 0.15 | 0.13 |
| Aluminum (ppm) | 9.49 | 4.17 | 7.39 |

Table 7.Soil's nutrients analysis after one growing season (fall 2018)

| Element | pH 5.0 | pH 6.5 | pH 8.0 |
|---|---|---|---|
| Organic matter (%) | 2.84 | 2.95 | 7.39 |
| pH | 5.69 | 6.05 | 8.88 |
| Moisture (%) | 2.09 | 1.80 | 1.36 |
| Phosphorus (%) | 2.16 | 1.16 | 6.66 |
| Potassium (%) | 19.58 | 25.91 | 26.11 |
| Calcium (%) | 3.46 | 3.54 | 5.69 |
| Magnesium (%) | 14.95 | 26.67 | 28.41 |
| Sulfur (%) | 6.81 | 1.96 | 1.22 |
| Zinc (ppm) | 2.13 | 1.08 | 1.04 |
| Manganese (ppm) | 1.86 | 3.85 | 1.57 |
| Iron (ppm) | 18.13 | 33.8 | 37.0 |
| Copper (ppm) | 0.50 | 0.31 | 0.12 |
| Boron (ppm) | 1.68 | 0.66 | 0.84 |
| Aluminum (ppm) | 5.78 | 5.62 | 10.01 |

### 3.6 Field growth performance

Tree's height & trunk diameter were measured twice during the growing season, once early in the spring and late fall. Tree height was measured using measuring tape from the soil line to the top point of the leader. The trunk diameter was measured using digital caliper from 15 centimeters above the drafting union. The difference in tree's height and TCSA were calculated between initial height and TCA in spring 2017 and the fall of 2017 representing the growth increase in the first year. The same was done for 2018 representing the height and TCSA growth in the second year. (Table 8)

*The first growing season 2017:*

The mean of the tree's height increase data in the first growing season shows no significant difference between rootstocks however, rootstock G.214 and M.9 showed the highest height difference with 37.8 and 36.9cm respectively in 2017. While the lowest increases were reported as 32.5 and 33.2 in rootstocks G.41 and B.9 respectively. A significant difference of $P \leq 0.05$ was observed in the mean tree's height increase in the first year within pH treatments. The highest mean of the height difference was 36.3 cm at pH 7.9 and the lowest was 33.0 cm at low pH. Regression analysis showed a linear relationship between soil pH and the tree's height increase in the first season. No significant difference was found in the interaction between the rootstock and the soil pH treatments in term of the means of the tree's height. However, rootstocks B.9, G.11, G.202, G214, and M.26 showed better height increase when grown in medium-range pH. On the other hand, rootstocks G.41 and M.9 showed highest height increases when grown in higher range pH, while G.935 has better tree's height at low pH range. (Table 8)

Comparing the difference of the trunk cross-section area (TCSA) of the first

growing seasons in 2017 also show a highly significant difference between rootstocks at P≤ 0.001. The highest increases in TCSA were 1.79 and 1.78 $cm^2$ in rootstock G.202 and M.26 respectively and the lowest was 0.59$cm^2$ in B.9. However, no significant difference was shown within pH treatments and the TCSA increase range was between 1.27 - 1.38$cm^2$. Also, no significant difference was found in the interaction between the rootstock and the soil pH treatments. However, TCSA data shows that rootstocks B.9, G.11, G.214, G.41, and G.935 had better increase at higher pH. While rootstocks M.9 and M.26 showed higher TCSA at medium pH whereas G.202 showed higher TCSA at low pH. (Table 8).

*The second growing season 2018:*

Overall, all rootstocks show less growth in terms of tree's height and TCSA compared to the first growing season. In some rootstock, the height differences were almost 50% less than the first season. However, a highly significant difference was found in the tree's height difference between rootstocks at P≤ 0.001. The highest increases were 35.7cm and 28.0cm in rootstocks G.935 and G.41. In the second year, no significant was reported in tree's height within pH treatments and the height increases were ranging from 22.5 cm to 24.4cm. No significant difference was found in the interaction between the rootstock and the soil pH treatments. However, rootstocks G.11, G.41, G214, and M.26 showed better height increase when grown in low pH. On the other hand, rootstocks G.935, G.202, and M.9 showed highest height increases when grown in medium pH while B.9 has better tree's height at high pH. (Table 8).

TCSA increase shows a highly significant difference at P≤ 0.001 between rootstocks with a maximum increase reported in rootstock G.935 by 2.96$cm^2$ and the

lowest TCSA increase was found in B.9 at 1.50cm. However, no significant difference was found in the pH treatments and the difference in TCSA was ranging from 2.09-2.68cm. Regression analysis showed a linear relationship between soil pH and TCSA increase in the second year. Regression analysis showed a linear relationship between soil pH and the TCSA increase in the second season. However, no significant difference was found in the interaction between the rootstock and the soil pH treatments in terms of TCSA in the second growing season. However, rootstocks G.11, G.41 G.202, G214, G.935, and M.26 showed better TCSA increase at high pH. While B.9 and M.9 showed higher TCSA at low pH (Table 8).

Overall, rootstock G.41 showed the lowest increase in tree's height during the first growing seasons. While rootstock B.9 showed the lowest TCSA in both growing seasons and the lowest tree's height in the second growing season. TCSA in both growing seasons within rootstocks. Rootstock G.214 shoed the highest increase in tree's height and G.202 showed the highest increase in TCSA during the first season. While in the second growing season, G.935 showed the highest height increase and the TCSA.

Table 8.Effect of soil pH on Honeycrisp tree growth of eight apple rootstocks in 2017 and 2018at Ithaca, NY.

| Rootstock | Soil pH | Tree height increase year 1 (cm) | TCSA increase year 1 ($cm^2$) | Tree height increase year 2 (cm) | TCSA increase year 2 ($cm^2$) |
|---|---|---|---|---|---|
| Main Effect | | | | | |
| B9 | . | 33.2 a [z] | 0.59 c | 14.1 d | 1.50 b |
| G11 | . | 33.8 a | 1.20 b | 18.0 cd | 1.66 b |
| G202 | . | 34.8 a | 1.79 a | 20.6 bcd | 2.71 a |
| G214 | . | 37.8 a | 1.60 ab | 17.7 cd | 2.67 a |
| G41 | . | 32.5 a | 0.73 c | 28.0 b | 1.98 ab |
| G935 | . | 34.4 a | 1.41 ab | 35.7 a | 2.96 a |
| M26 | . | 36.1 a | 1.78 a | 26.9 b | 2.91 a |
| M9 | . | 36.9 a | 1.48 ab | 25.3 bc | 2.03 ab |
| Rootstock significance | | NS | *** | *** | *** |
| - | Low | 33.0 b | 1.27 a | 24.41a | 2.09 a |
| - | medium | 35.6 ab | 1.38 a | 22.50 a | 2.24 a |
| - | high | 36.3 a | 1.32 a | 23.19 a | 2.68 a |
| pH (regression) | | L* | NS | NS | L* |
| Interaction means | | | | | |
| B9 | Low | 31.8 | 0.48 | 13.8 | 1.60 |
| | medium | 34.3 | 0.62 | 13.3 | 1.51 |
| | high | 33.5 | 0.67 | 17.2 | 1.23 |
| G11 | Low | 30.7 | 1.13 | 19.9 | 1.53 |
| | medium | 36.7 | 1.24 | 17.2 | 1.65 |
| | high | 34.1 | 1.26 | 17.6 | 1.76 |
| G202 | Low | 31.3 | 2.00 | 20.9 | 2.54 |
| | medium | 37.4 | 1.75 | 22.0 | 2.64 |
| | high | 35.6 | 1.63 | 19.2 | 2.89 |
| G214 | Low | 32.2 | 1.41 | 22.6 | 1.31 |
| | medium | 41.9 | 1.55 | 16.6 | 2.95 |
| | high | 39.3 | 1.84 | 14.4 | 3.61 |
| G41 | Low | 29.1 | 0.54 | 25.4 | 1.99 |
| | medium | 33.3 | 0.75 | 24.4 | 1.92 |
| | high | 35.0 | 0.89 | 38.3 | 2.09 |
| G935 | Low | 36.3 | 1.44 | 32.3 | 2.79 |
| | medium | 30.4 | 1.53 | 35.9 | 2.61 |
| | high | 35.1 | 1.70 | 18.9 | 3.05 |
| M26 | Low | 33.6 | 1.75 | 36.2 | 2.88 |
| | medium | 39.5 | 1.89 | 27.2 | 2.77 |
| | high | 35.1 | 1.70 | 18.9 | 3.05 |
| M9 | Low | 38.5 | 1.40 | 25.1 | 2.08 |
| | medium | 30.9 | 1.67 | 26.6 | 1.97 |
| | high | 41.3 | 1.36 | 24.2 | 2.06 |
| Interaction significance | | NS | NS | NS | NS |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a nonsignificant effect, respectively.

### 3.7 Leaf nutrients analysis

**Leaf carbon and Nitrogen contents**

*The first growing season 2017:*

There was a highly significant difference in carbon content percentage in the first growing season within the tested apple rootstocks at P≤ 0.001. Also, a highly significant difference in Carbon content percentage in the first growing season within soil pH treatments at P≤ 0.001 and a quadratic relationship within soil pH and leaf carbon content during the first growing season. There was a highly significant difference in carbon content percentage in the first growing season within the interaction between soil pH and rootstock at P≤ 0.001. (Table 9)

The highest mean of a leaf's carbon content in the first year was recorded in rootstock G.214 at 47.1% followed by M.26 at 46.9%. While the slightly lower contents were shown in rootstock M.9 at 46.4%. Rootstocks G.202, G.41, G214, and M.9 showed better leaf carbon content when grown in medium pH. In the other hand, rootstocks B.9, G.935, and M.26 showed leaf carbon content when grown in high pH while G11 has better tree's height at low pH. (Table 9)

The mean of a leaf's nitrogen content in the first year shows a significant difference in nitrogen content percentage in the first growing season within the rootstock at P≤ 0.05. Rootstocks G.202 was reported the highest nitrogen percentage of 2.40 % among all rootstocks. While M.9 showed the lowest nitrogen contents at 1.9 % in the first growing season. However, no significant difference in nitrogen content percentage in the first growing season within the pH treatments and no significant difference were found in the interactions between rootstocks and soil pH. However, rootstocks B.9, G.935, and M.9 showed higher leaf nitrogen content at high pH, while rootstocks G.11, G.202, G.41,

and M.26 showed higher percentage at low pH. (Table 9)

*The second growing season 2018:*

The carbon content percentage in the second growing season found to be a significant difference within the tested apple rootstocks at P≤ 0.005. However, no significant difference was shown within soil pH treatments or within the interaction between soil pH and rootstock. (Table 9)

The highest mean of a leaf's carbon content in the second year was recorded in rootstock B.9 at 48.1% followed by G.41 at 48.0%. While all other rootstock showed lower contents range from 46.8- 47.8%. Rootstocks G.11, G.202, G214, G.935, and M.9 showed better leaf carbon content when grown in high pH. However, rootstocks B.9, G.41, and M.26 showed leaf carbon content when grown in low pH while G11 has better tree's height at low pH. (Table 9)

The mean of a leaf's nitrogen content in the second growing season shows a significant difference in nitrogen content percentage within the rootstock at P≤ 0.05. Rootstocks G.41 was reported the highest nitrogen percentage of 2.19 % among all rootstocks. While G.214 and M.26 showed the lowest nitrogen contents at 1.99 % in the second growing season. However, no significant difference in nitrogen content percentage in the second growing season within the pH treatments and no significant difference were found in the interactions between rootstocks and soil pH. Rootstocks B.9 and G.935 showed higher leaf nitrogen content at low pH, while rootstocks G.202, G.41, M.9, and M.26 showed higher percentage at high pH. Interestingly, rootstock G.11 show a similar value of leaf nitrogen in both high and low pH. (Table 9).

**Leaf macro and micronutrients analysis.**

*The first growing season 2017:*

A highly significant difference was found in all macronutrients mean percentage at P≤ 0.001 in the first growing season within the tested apple rootstocks. Also, a highly significant difference was shown in the manganese concentration at P≤ 0.001and a significant difference was shown in the zinc at P≤ 0.005. However, iron and boron showed no significant difference within rootstocks in the first growing season. (Table.10).

The nutrient analysis showed that rootstock G.11 was found to have better values of phosphorus, iron, and manganese while M.26 was better in potassium and calcium values. G.2020 was noted higher values of magnesium and zinc.

Similarly, a highly significant difference was found in all macronutrients and zinc mean percentage at P≤ 0.001 in the first growing season in response to soil pH. A significant difference was shown in manganese concentration at P≤ 0.005 within soil treatments. While no significant difference was found in iron and boron in response to soil pH. Strong quadratic relationships were found in phosphorus, magnesium, sulfur, and zinc within soil pH treatments. Whereas, linear relationships were found in potassium and manganese under soil pH treatments. (Table.10).

A highly significant difference at P≤ 0.001was found in phosphorus, magnesium, and sulfur in the interaction between rootstocks and soil pH. and a significant difference at P≤ 0.005 was found in potassium, calcium, and zinc in the interaction between rootstocks and soil. Whereas, no significant difference was found in boron, iron or Manganese. All macronutrients values were found higher at high soil pH in all rootstocks. Zinc and iron values were higher in rootstocks G.11, G.202 and G.214 at low pH while in B.9 values were higher in high pH. Manganese values were higher at high pH in all rootstocks. (Table.10).

*The second growing season 2018:*

The second-year leaf nutrients analysis found to be the highly significant difference at P≤ 0.001 in calcium and boron content and a significant difference at P≤ 0.005 in potassium and zinc within rootstocks. A significance difference at P≤ 0.05 was also found in magnesium however, no significant difference was shown in sulfur, iron or manganese in all tested rootstocks. The data showed that rootstock G.935 had higher values of calcium, magnesium, sulfur, iron, and manganese among all rootstocks in the second growing season. Rootstock G.202 had also higher contents of potassium and boron while G.41 had shown higher values of phosphorus and zinc. (Table.11).

Under pH treatments, a highly significant difference at P≤ 0.001was found in manganese within pH treatments. Also, a significant difference at P≤ 0.05 was shown in magnesium, sulfur and boron potassium, and iron in response to soil pH. The regression analysis showed a quadratic relationship between pH and potassium and iron ad linear relationships in magnesium, sulfur, and manganese. (Table.11).

In the second-year leaf nutrients analysis showed different patterns than those in the first year. However, like the first season, values of phosphorus were found higher at soil high pH in all rootstocks and the highest in M.9. No other pattern was noted in the higher nutrient values distribution among pH treatments. The interaction effect between rootstocks and pH treatments was noted significant phosphorus, magnesium, sulfur, iron, and manganese. However, no significant differences were found in phosphorus, potassium calcium boron and zinc. (Table.11).

Individual leaf nutrients by rootstock show different pattern in response to soil pH. (Appendix figures 18-28)

The cell plot in figure 6 shows leaf nutrients concentrations of P, K, Ca, Mg, S, B, Fe, Mn and Zn from each rootstock in response to pH treatments from the second season of 2018. Concentration is presented in color patterns. Red represents higher concentration and blue present lower concentration. Each bar set denotes pH treatment (5.0, 6.5 and 8.0). From this nutrient analysis, lower nutrient concentrations are shown in almost all rootstock growing in alkaline soil.

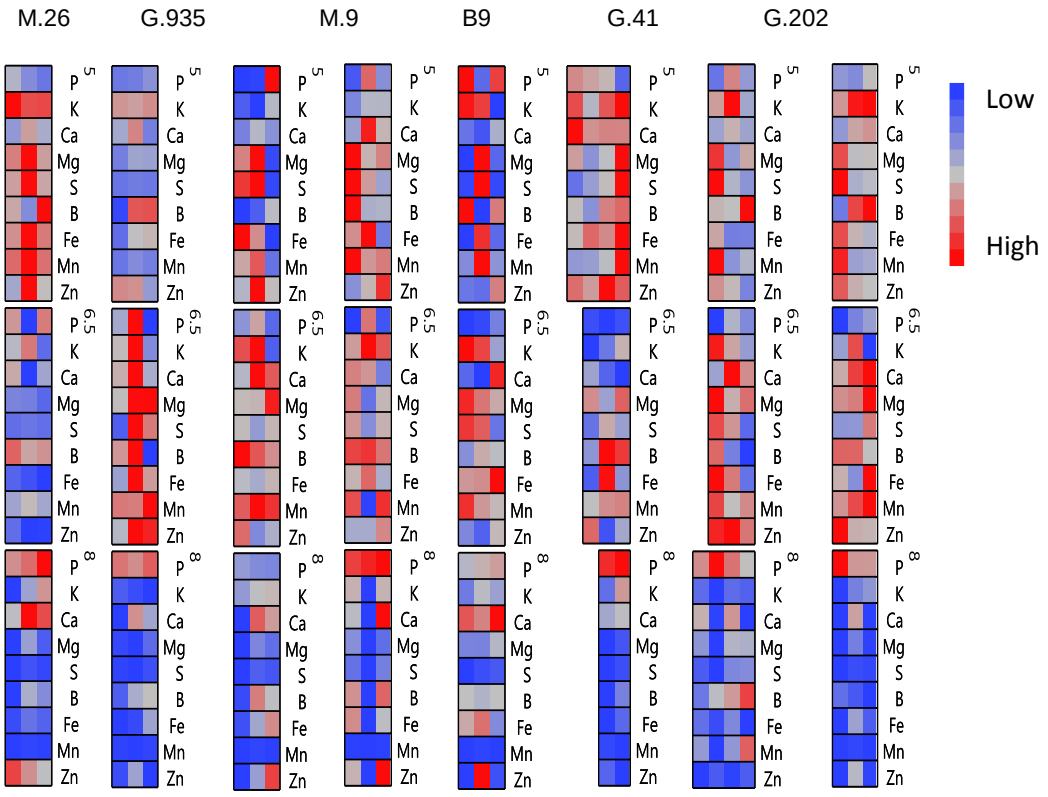


Figure 7. Cell plot of Leaf nutrients concentration from each rootstock in response to soil pH.

Table 9.Effect of soil pH on Honeycrisp leaf nutrients concentration of eight apple rootstocks in 2017 at Ithaca

| Rootstock | Soil pH | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | | | | | |
| B9 | . | 0.77b [z] | 1.01bc | 3.61a | 4.61ab | 0.75c | 0.73a | 11.43cd | 12.7ab | 26.7bc | 74.9bc |
| G11 | . | 1.0a | 1.22a | 3.37a | 3.67c | 1.01a | 0.69ab | 9.87d | 9.0b | 25.4bcd | 95.8ab |
| G202 | . | 0.81b | 1.01bc | 3.35a | 4.70ab | 1.02a | 0.66bc | 12.67c | 18.4a | 32.4a | 82.4bc |
| G214 | . | 0.59c | 0.81d | 2.86b | 4.48ab | 0.61d | 0.46e | 16.49a | 12.1b | 22.5cde | 73.1bc |
| G41 | . | 0.8b | 1.05b | 2.75b | 4.36b | 0.60d | 0.62cd | 16.43a | 13.4b | 21.0cde | 65.9c |
| G935 | . | 0.74bc | 0.93c | 2.88b | 5.03a | 0.86b | 0.60cd | 12.50c | 12.3b | 24.6bcde | 40.9d |
| M26 | . | 0.74bc | 0.97bc | 3.72a | 3.75c | 0.76bc | 0.59d | 10.07d | 11.2b | 19.5e | 95.2ab |
| M9 | . | 0.75b | 1.02bc | 3.51a | 4.70ab | 0.81bc | 0.56d | 14.33b | 13.2b | 28.2ab | 112a |
| Rootstock significance | | *** | *** | *** | | *** | *** | *** | ** | *** | *** |
| - | Low | 0.8a | 0.95b | 2.78c | 4.2b | 0.69b | 0.71a | 11.2c | 14.2a | 32.4a | 65.9b |
| - | medium | 0.73b | 0.90b | 3.11b | 4.6a | 0.73b | 0.56b | 13.2b | 9.1b | 18.4c | 81.4a |
| - | high | 0.8a | 1.15a | 3.88a | 4.4ab | 0.99a | 0.58b | 14.4a | 15.2a | 24.2b | 93.1a |
| pH significance | | *** | *** | *** | | *** | *** | *** | *** | *** | ** |
| Regression | | Q** | L*** | L*** | | Q** | Q** | Q** | Q*** | Q** | L** |

Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels, or had a nonsignificant effect, respectively

Table 10.Effect of the interaction of soil pH on Honeycrisp leaf nutrients concentration of eight apple rootstocks in 2017 at Ithaca

| Rootstock | Soil pH | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Interaction means | | | | | | | | | | | |
| B9 | Low | 0.75fedg [Z] | 0.88jifgh | 3.5dce | 4.04hjfkig | 0.55klj | 0..84ba | 12.31fe | 13.2fhdeicg | 38.92ba | 73.3fged |
| | medium | 0.70fhg | 0.85jigh | 3.1hdfge | 3.81hjkig | 0.56klj | 0.68edc | 10.86gifh | 8.7hjig | 13.90g | 73.3fged |
| | high | 0.87bdc | 1.31ba | 4.3ba | 5.98a | 1.13bc | 0.68edc | 11.13gfh | 16.4fbdec | 27.21dc | 78.2fed |
| G11 | Low | 1.05a | 1.20bcd | 2.8h | 3.34jk | 0.85def | 0.74bdc | 8.82ih | 11.5fhdjeig | 31.49 bdac | 68.9fged |
| | medium | 0.94bdc | 1.20bcd | 3.1 dfge | 3.47jki | 0.96dc | 0.62egf | 9.51gih | 5.2j | 17.8feg | 93.8fbedc |
| | high | 1.02a | 1.24bcd | 4.2ba | 4.20ehjfkig | 1.21ba | 0.72edc | 11.28gfh | 10.4fhjei | 26.79dc | 124.8ba |
| G202 | Low | 0.82fedg | 0.95efgh | 2.9hjfgei | 4.84ebdfc | 0.87def | 0.72edc | 11.03gfh | 24.9a | 39.65a | 67.9fged |
| | medium | 0.76fedg | 0.98efdh | 2.9hjfgei | 4.52ehdfcg | 0.86def | 0.63edf | 11.54gfh | 12.9fhdeicg | 24.26fde | 77.7fed |
| | high | 0.83fedg | 1.11ecd | 4.2ba | 4.75ebdfcg | 1.32a | 0.64edf | 15.44dc | 17.4bdec | 33.18bac | 101.8bdc |
| G214 | Low | 0.66ihg | 0.80jih | 2.5ji | 4.45ehdfig | 0.68ihgklj | 0.55hgf | 13.59dfe | 15.6fbdecg | 33.32bac | 59.1fgeh |
| | medium | 0.59ih | 0.75ji | 3.1hdfge | 5.03ebdac | 0.55kl | 0.43ij | 19.20ba | 8.7hjig | 16.42fg | 77.6fed |
| | high | 0.54i | 0.90ifgh | 3.0hdfgei | 3.96hjfkig | 0.6ihklj | 0.40j | 16.69bc | 12.0fhdjeicg | 17.82feg | 82.5fedc |
| G41 | Low | 0.95bac | 1.19bcd | 2.5hji | 4.20ehjfkig | 0.57iklj | 0.84a | 15.26dc | 19.0bac | 31.25bdc | 59.5fged |
| | medium | 0.75fedg | 0.9ifgh | 2.8hjfgi | 5.42bdac | 0.51l | 0.53higf | 14.55dce | 9.4fhjig | 17.30fg | 71.1fgeh |
| | high | 0.69fhg | 1.07efcd | 2.9hdfgei | 3.47jk | 0.71ihgkfj | 0.49hij | 19.4a | 12.0fhdjeicg | 14.36g | 67.1fged |
| G935 | Low | 0.77fedg | 0.87jigh | 2.3j | 4.06ehjfkig | 0.72ihgefj | 0.72edc | 11.25gfh | 6.6ji | 27.98dc | 27.4h |
| | medium | 0.71fhg | 0.82jih | 2.7hjgi | 5.55ba | 0.89de | 0.52hig | 14.53dce | 9.3fhjig | 17.65feg | 38.9gh |
| | high | 0.74fedg | 1.10ecd | 3.6dc | 5.49bac | 0.97dc | 0.56hgf | 11.72gf | 21.1ba | 28.13dc | 56.3fgh |
| M26 | Low | 0.86bedc | 1.06efcd | 3.0hdfgei | 4.25ehjfig | 0.71ihgkfj | 0.77bac | 8.12i | 13.0fhdeicg | 25.94dec | 91.4fbedc |
| | medium | 0.64ihg | 0.83jih | 3.8bc | 3.75hjki | 0.73ihgef | 0.49hij | 11.22gfh | 6.9hji | 14.26g | 99.7bdc |
| | high | 0.72fehg | 1.04efgd | 4.3ba | 3.24k | 0.85dgef | 0.51hij | 10.87gifh | 13.7fhdecg | 18.28feg | 94.5bedc |
| M9 | Low | 0.54i | 0.70j | 2.7hjgi | 4.43ehdfig | 0.59ihklj | 0.49hij | 9.43gih | 9.5fhjig | 30.83bdc | 79.3fed |
| | medium | 0.72fehg | 0.90ifgh | 3.3dfce | 5.41bdac | 0.76 hgef | 0.57hgf | 14.59dce | 12.0fhdjeicg | 25.79dec | 118.9bac |
| | high | 0.99ba | 1.46a | 4.5a | 4.26ehjfig | 1.09 bc | 0.63ef | 18.97ba | 18.3bdac | 28.12dc | 139.4a |
| Interaction significance | | ** | *** | ** | *** | *** | *** | *** | ** | * | * |

[Z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non significant effect, respectively.

Table 11. Effect of soil pH on Honeycrisp leaf nutrients concentration of eight apple rootstocks in 2018 at Ithaca.

| Rootstock | Soil pH | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | | | | | |
| B9 | . | 0.77b [z] | 1.33ab | 0.94d | 1.58a | 0.27bc | 0.20a | 24.9d | 18.1a | 67.9b | 15.9a |
| G11 | . | 1.00a | 1.32ab | 1.00cd | 1.23bc | 0.33ab | 0.21a | 28.1c | 13.6bc | 72.4ab | 20.1a |
| G202 | . | 0.81b | 1.38ab | 1.18a | 0.94d | 0.25bc | 0.20a | 33.8a | 12.5c | 70.3b | 13.1a |
| G214 | . | 0.59c | 1.15b | 1.09abc | 1.24bc | 0.23c | 0.21a | 31.2b | 13.3bc | 70.3b | 15.0a |
| G41 | . | 0.80b | 1.49a | 1.04bcd | 1.32b | 0.32ab | 0.25a | 28.4c | 19.1a | 76.9ab | 13.8a |
| G935 | . | 0.74bc | 1.44a | 1.12ab | 1.66a | 0.38a | 0.33a | 29.5bc | 17.5ab | 92.2a | 23.4a |
| M26 | . | 0.74bc | 1.30ab | 1.05bcd | 1.05cd | 0.33ab | 0.23a | 24.5d | 13.5ab | 88.5ab | 18.4a |
| M9 | . | 0.75b | 1.51a | 1.02bcd | 1.24bc | 0.33ab | 0.24a | 25.4d | 16.7abc | 71.7ab | 19.3a |
| Rootstock significance | | *** | * | ** | *** | * | NS | *** | ** | NS | NS |
| - | Low | 0.80a | 1.34a | 1.07a | 1.29a | 0.34a | 0.26a | 28.9a | 15.6a | 78.0a | 24.9a |
| - | medium | 0.73b | 1.31a | 1.10a | 1.27a | 0.33a | 0.27a | 29.1a | 16.3a | 84.1a | 20.7a |
| - | high | 0.80a | 1.43a | 0.99b | 1.29a | 0.24b | 0.17b | 26.7b | 14.6a | 66.4b | 60.9b |
| pH significance | | ** | NS | * | NS | ** | ** | ** | NS | * | *** |
| Regression | | Q* | NS | Q* | NS | L** | L* | * | NS | Q* | L*** |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non significant effect, respectively.

Table 12.Effect of the interaction of soil pH on Honeycrisp leaf nutrients concentration of eight apple rootstocks in 2018 at Ithaca

| Rootstock | Soil pH | N (%) | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Interaction means | | | | | | | | | | | |
| B9 | Low | 0.75fcde [z] | 1.30 | 1.0 | 1.5 | 0.35cbd | 0.25abce | 25.5 | 19.7 | 71.0ecd | 25.3cb |
| | medium | 0.70fgh | 1.28 | 0.92 | 1.7 | 0.27fed | 0.20abce | 25.7 | 16.9 | 69.8ecd | 19.8cbd |
| | high | 0.87bcd | 1.41 | 0.90 | 1.5 | 0.19fe | 0.14ace | 23.4 | 17.7 | 63.1ed | 2.5e |
| G11 | Low | 1.05a | 1.28 | 0.98 | 1.3 | 0.36cbd | 0.28abce | 27.5 | 12.6 | 79.4ecd | 24.0cb |
| | medium | 0.94abc | 1.21 | 1.05 | 1.3 | 0.42cb | 0.22abce | 31.6 | 15.3 | 81.5ecd | 34.5b |
| | high | 1.02a | 1.46 | 0.96 | 1.1 | 0.20fe | 0.13a | 25.3 | 13.0 | 56.5ed | 1.8e |
| G202 | Low | 0.82cdef | 1.38 | 1.15 | 0.8 | 0.27fed | 0.24abce | 35.5 | 12.5 | 77.8ecd | 15.9ced |
| | medium | 0.76defg | 1.16 | 1.26 | 1.1 | 0.27fed | 0.19ace | 33.7 | 13.9 | 69.5ecd | 16.9ced |
| | high | 0.83cdef | 1.70 | 1.13 | 0.9 | 0.20fe | 0.14ace | 30.4 | 10.4 | 56.5ed | 1.9ee |
| G214 | Low | 0.66hig | 1.11 | 1.06 | 1.4 | 0.24fed | 0.25abce | 30.2 | 15.2 | 66.5ecd | 16.7ced |
| | medium | 0.59hi | 1.03 | 1.21 | 1.2 | 0.27fed | 0.23abce | 32.5 | 14.5 | 94.4bc | 18.7cbd |
| | high | 0.54i | 1.26 | 1.02 | 1.2 | 0.18f | 0.15ace | 30.9 | 11.0 | 55.0e | 11.0ed |
| G41 | Low | 0.95abc | 1.72 | 1.11 | 1.2 | 0.30ced | 0.26abce | 27.7 | 17.0 | 66.6ecd | 18.1cbd |
| | medium | 0.75defg | 1.22 | 1.04 | 1.1 | 0.37cbd | 0.33bcde | 29.2 | 18.9 | 87.3bcd | 21.8cbd |
| | high | 0.69fgh | 1.53 | 0.97 | 1.6 | 0.29fed | 0.16ace | 28.1 | 21.6 | 76.8ecd | 1.4e |
| G935 | Low | 0.77defg | 1.36 | 1.18 | 1.9 | 0.31ced | 0.21abce | 30.3 | 21.0 | 85.5bcd | 11.7ced |
| | medium | 0.71fgh | 1.48 | 1.17 | 1.7 | 0.59a | 0.57d | 30.3 | 17.5 | 126.6ba | 51.3a |
| | high | 0.74defg | 1.62 | 1.01 | 1.5 | 0.21fe | 0.14ace | 27.9 | 13.8 | 63.9ecd | 2.2e |
| M26 | Low | 0.86bcde | 1.19 | 1.02 | 0.9 | 0.45b | 0.40bd | 25.3 | 12.1 | 132.2a | 35.5b |
| | medium | 0.64ghi | 1.25 | 1.15 | 1.0 | 0.28 fed | 0.17ace | 25.1 | 14.2 | 65.1ecd | 16.6ced |
| | high | 0.72efgh | 1.46 | 0.98 | 1.2 | 0.27fed | 0.14ac | 23.0 | 14.3 | 68.3ecd | 3.2e |
| M9 | Low | 0.54i | 1.72 | 1.06 | 1.4 | 0.37cbd | 0.34bde | 27.0 | 15.5 | 76.5ecd | 20.7cbd |
| | medium | 0.72efgh | 1.45 | 0.99 | 1.1 | 0.39cbd | 0.24abce | 24.5 | 19.4 | 71.0ecd | 34.8b |
| | high | 0.99ab | 1.35 | 1.01 | 1.2 | 0.21fe | 0.14ace | 24.6 | 15.2 | 67.7ecd | 2.4e |
| Interaction significance | | *** | NS | NS | NS | * | * | NS | NS | * | ** |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non significant effect, respectively.

Table 13.Average yield per tree (fruit) from eight rootstocks under soil pH treatments in 2018.

| Rootstock | pH | Fruit NO. |
|---|---|---|
| B.9 | Low | 9.5 |
| B.9 | medium | 10.1 |
| B.9 | high | 6.6 |
| G.11 | Low | 6.8 |
| G.11 | medium | 5.9 |
| G.11 | high | 4.7 |
| G.202 | Low | 3 |
| G.202 | medium | 4.3 |
| G.202 | high | 3.3 |
| G.214 | Low | 6.8 |
| G.214 | medium | 9.1 |
| G.214 | high | 5.8 |
| G.41 | Low | 10.3 |
| G.41 | medium | 8.6 |
| G.41 | high | 10.3 |
| G.935 | Low | 8.6 |
| G.935 | medium | 5.7 |
| G.935 | high | 7.1 |
| M.26 | Low | 3.6 |
| M.26 | medium | 6 |
| M.26 | high | 3.7 |
| M.9 | Low | 6.8 |
| M.9 | medium | 4.8 |
| M.9 | high | 3.5 |

### 3.1 Fruit's maturity parameters

Fruit quality was only assessed and evaluated during the second growing season in 2018. Fruit parameters measured by the fruit sorting machine provided clear and accurate measurements. All fruits from each tree were run through the sorting machine separately. Fruit weight and skin color showed a significant difference within rootstocks. A highly significant difference at $P \leq 0.001$was found in fruit skin green color percentage in all rootstocks. Also, significant differences were reported in the fruit's weight, and skin color red and yellow. However, no significant difference was found in the fruit's size and fruit length (Table 14).

Rootstock G.11 showed a bigger fruit size, weight, and length while M.9 showed better red skin color percentage among rootstocks. All fruit maturity parameters except for yellow and red skin showed a significant difference in all soil pH treatments. However better values of fruit's weight, size, and length were found at high pH within pH treatments. A strong linear relationship was found in fruit's weight, size and length in response to soil pH.

With exception to G.11, all rootstock had the highest fruit's weight and size at high pH scoring maximum average weight of 228g and average size 82.1mm per fruit. G.11 showed better fruit weight, size, and length at medium pH. Fruit's length was also higher in high pH in rootstock B.9, G.214, G, 41, G.935, and M.9.

Red skin color was also improved at high pH in rootstocks B.9, G.202, G.214 while it was higher in medium pH in rootstocks G.41, G.935, and M.26 (Table 14).

Comparing the mean of the average red skin color percentage of Honeycrisp™ apple shows no significant differences within pH treatments but there was a significant

difference between rootstocks (Table 14).

The interaction between rootstocks and soil pH treatments were significant in fruit length and skin color green and red however not significant in fruit's weight, size or yellow skin color.


Figure 8 Fruits from G.41 rootstock from three pH treatments. (8.0, 6.5, 5.0).


Figure 9. Fruits from.M.26 rootstock from three pH treatments. (7.9, 4.3, 6.7).

**3.2 Fruit quality and storage disorders assessments.**

The rootstock effect on fruit quality and storage disorders was only significant on yield at P≤ 0.001. However, no significant difference was found in total soluble solids %, fruit's firmness, yield, bitter-pit incident percentage. The highest fruit per tree was found produced by rootstock G.41 under pH experiment. And the lowest bitter-pit % was reported in G.935(Table 15).

Soil pH treatments showed a significant difference in total soluble solids %, fruit's firmness, and bitter pit. A strong linear relationship was also found in total soluble solids %, fruit's firmness and a strong quadratic relationship in the bitter-pit incident percentage. Total soluble solids % and firmness were found following the same patterns as the highest values were reported in low pH treatment in rootstocks G.11, G.202, G.214, M9, and M.26 whereas in medium pH in rootstocks B.9, G.41, and G.935. The highest yield was noted in rootstocks G.41 and B.9 with an average mean of 10.33 in high pH and 10.11 fruit per tree in medium pH respectively. The highest bitter-pit incident was found in rootstock G.11 at high pH and no incident was found in B.9, G.214, and G.935 at low pH and in G.202 in medium pH. The soggy-breakdown disorder was also higher high pH in rootstocks B.9, G.202, G.214, G.935, and M.26 and lower in low pH in rootstocks G.202, G.214, G.41, and M.26 (Table 15).

No significant difference was found in the interaction between rootstocks and soil pH in all fruit quality and storage disorder. Due to the low yield per tree on some trees, data may not be representing the effect of rootstock and pH precisely. However, when assessing individual rootstock in regression analysis, strong correlations were found. (Appendix tables 30-53)

Table 14.Effect of soil pH on Honeycrisp fruit parameters of eight apple rootstocks in 2018

| Rootstock | Soil pH | Fruit weight (g) | Fruit size (mm) | Fruit Length (mm) | Skin color Green (%) | Skin color Yellow(%) | Skin color Red (%) |
|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | |
| B9 | . | 173a[Z] | 72.a | 74.6ab | 1.95b | 36.3a | 61.4ab |
| G11 | . | 195a | 74.4a | 77.5ab | 1.48b | 35.7a | 62.4ab |
| G202 | . | 194a | 74.7a | 73.8ab | 0.80b | 38.4a | 65.5ab |
| G214 | . | 185a | 73.1a | 76.1ab | 0.85b | 30.6a | 68.1ab |
| G41 | . | 162a | 69.8a | 72.3ab | 9.05a | 41.6a | 49.2b |
| G935 | . | 172a | 73.7a | 67.4b | 0.89b | 34.1a | 77.7a |
| M26 | . | 202a | 74.7a | 77.9a | 0.37b | 31.8a | 65.2ab |
| M9 | . | 182a | 72.2a | 75.4ab | 0.61b | 28.3a | 69.8ab |
| Rootstock significance | | * | NS | NS | *** | NS | NS |
| - | Low | 151b | 68.1c | 77.7b | 3.47a | 35.9a | 60.4ab |
| - | medium | 181b | 72.7b | 73.0b | 1.40b | 32.4a | 70.2a |
| - | high | 217a | 77.5a | 79.7a | 1.12b | 35.5a | 64.4ab |
| pH significance | | ** | ** | ** | ** | NS | NS |
| Regression | | L** | L*** | L** | L* | NS | NS |
| Interaction means | | | | | | | |
| B9 | Low | 127 | 64.6 | 66.9ab | 1.19b | 36.5 | 62.3 |
| | medium | 159 | 70.3 | 73.6ab | 3.95b | 43.2 | 52.9 |
| | high | 235 | 79.7 | 83.4a | 0.70b | 29.3 | 69.2 |
| G11 | Low | 157 | 69.2 | 72.1ab | 0.54b | 33.6 | 65.9 |
| | medium | 234 | 79.0 | 82.5a | 1.11b | 26.4 | 71.3 |
| | high | 190 | 75.1 | 78.0a | 2.79b | 47.2 | 50.0 |
| G202 | Low | 158 | 70.7 | 72.8ab | 0.50b | 35.2 | 64.3 |
| | medium | 197 | 75.2 | 78.2a | 1.01b | 37.8 | 61.2 |
| | high | 230 | 78.2 | 70.5ab | 0.90b | 42.2 | 70.8 |
| G214 | Low | 168 | 69.8 | 72.3ab | 0.56b | 32.1 | 65.9 |
| | medium | 177 | 72.3 | 75.5a | 1.75b | 32.5 | 65.8 |
| | high | 208 | 77.3 | 80.4a | 0.25b | 27.2 | 72.5 |
| G41 | Low | 110 | 61.7 | 63.8ab | 22.21a | 52.2 | 25.5 |
| | medium | 159 | 69.2 | 72.3ab | 1.80b | 36.4 | 61.3 |
| | high | 218 | 78.5 | 80.9a | 3.15b | 36.2 | 60.6 |
| G935 | Low | 155 | 68.2 | 71.3ab | 1.15b | 37.0 | 61.9 |
| | medium | 158 | 70.5 | 50.8b | 0.91b | 28.1 | 108.9 |
| | high | 204 | 76.5 | 80.0a | 0.60b | 37.2 | 62.2 |
| M26 | Low | 173 | 70.7 | 73.6a | 0.21b | 34.0 | 62.7 |
| | medium | 207 | 77.0 | 80.5a | 0.53b | 28.4 | 71.1 |
| | high | 226 | 76.4 | 79.7a | 0.36b | 33.1 | 62.0 |
| M9 | Low | 162 | 69.8 | 73.0ab | 1.40b | 26.6 | 72.0 |
| | medium | 160 | 68.0 | 71.1ab | 0.17b | 26.8 | 69.4 |
| | high | 224 | 78.7 | 82.0a | 0.25b | 31.6 | 68.1 |
| Interaction significance | | NS | NS | * | *** | NS | NS |

[Z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non significant effect, respectively.

Table 15. Effect of soil pH on Honeycrisp fruit quality and storage disorders on of eight apple rootstocks in 2018

| Rootstock | Soil pH | TSS (°Bx) | Firmness (kg/cm$^2$) | Fruit NO. | Bitter pit (%) | Soggy breakdown (%) |
|---|---|---|---|---|---|---|
| Main Effect | | | | | | |
| B9 | . | 14.6 ab [z] | 6.63ab | 8.74 a | 0.10a | 0.07a |
| G11 | . | 14.5 b | 6.48ab | 5.88 cd | 0.25a | 0.09a |
| G202 | . | 14.7 ab | 6.86ab | 3.71 d | 0.14a | 0.07a |
| G214 | . | 15.3 a | 7.29a | 7.50ab | 0.08a | 0.14a |
| G41 | . | 15.2 ab | 7.03ab | 9.20 a | 0.19a | 0.09a |
| G935 | . | 14.7 ab | 6.76ab | 6.83 abc | 0.07a | 0.16a |
| M26 | . | 14.9 ab | 6.26b | 4.55 cd | 0.19a | 0.09a |
| M9 | . | 15 ab | 6.74ab | 4.78 cd | 0.11a | 0.10a |
| Rootstock significance | | NS | NS | *** | NS | NS |
| - | Low | 15.2 a | 7.06 a | 6.93 a | 0.12 b | 0.06 b |
| - | medium | 15.0 a | 6.84ab | 6.97 a | 0.08 b | 0.10ab |
| - | high | 14.3 b | 6.33b | 5.46 a | 0.24 a | 0.14a |
| pH significance | | ** | * | NS | ** | NS |
| Regression | | L*** | L** | NS | Q** | NS |
| Interaction means | | | | | | |
| B9 | Low | 14.48 | 6.88 | 9.50 | 0.00 | 0.04 |
| | medium | 14.99 | 7.27 | 10.11 | 0.00 | 0.04 |
| | high | 14.39 | 5.73 | 6.63 | 0.30 | 0.13 |
| G11 | Low | 14.95 | 7.15 | 6.75 | 0.16 | 0.06 |
| | medium | 14.16 | 5.99 | 5.89 | 0.23 | 0.13 |
| | high | 14.90 | 7.05 | 4.67 | 0.44 | 0.00 |
| G202 | Low | 15.20 | 7.75 | 3.00 | 0.17 | 0.00 |
| | medium | 14.85 | 6.87 | 4.33 | 0.00 | 0.08 |
| | high | 14.30 | 6.56 | 3.33 | 0.26 | 0.08 |
| G214 | Low | 15.53 | 7.65 | 6.75 | 0.00 | 0.06 |
| | medium | 15.26 | 7.05 | 9.13 | 0.06 | 0.20 |
| | high | 14.70 | 7.05 | 5.75 | 0.28 | 0.15 |
| G41 | Low | 14.58 | 7.12 | 10.25 | 0.38 | 0.07 |
| | medium | 15.63 | 7.17 | 8.62 | 0.09 | 0.08 |
| | high | 14.30 | 6.31 | 10.33 | 0.38 | 0.20 |
| G935 | Low | 15.08 | 6.72 | 8.60 | 0.00 | 0.11 |
| | medium | 15.27 | 7.16 | 5.57 | 0.05 | 0.04 |
| | high | 14.26 | 6.55 | 7.08 | 0.11 | 0.21 |
| M26 | Low | 15.80 | 6.40 | 3.63 | 0.26 | 0.00 |
| | medium | 15.05 | 6.28 | 6.00 | 0.11 | 0.12 |
| | high | 13.90 | 6.13 | 3.70 | 0.24 | 0.13 |
| M9 | Low | 15.78 | 7.40 | 6.75 | 0.07 | 0.10 |
| | medium | 14.91 | 7.01 | 4.75 | 0.04 | 0.12 |
| | high | 14.60 | 5.94 | 3.50 | 0.23 | 0.08 |
| Interaction significance | | NS | NS | NS | NS | NS |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non significant effect, respectively.

### 3.1 Fruit peel nutrients analysis

The peel nutrients analysis found to be a highly significant difference at $P \leq 0.001$ in boron and significant difference at $P \leq 0.005$ in phosphorus, potassium, calcium, and magnesium within all tested rootstocks. However, sulfur, zinc, iron, and manganese showed no significant difference within rootstocks. Fruit peel nutrients analysis did show any pattern in rootstock partitioning for more than one element. However, the average phosphorus percentage was higher in rootstock G.202 and potassium was higher in M.9 while calcium was higher in B.9 (Table 16).

Statistical analysis showed that soil pH treatment affects fruit's peel nutrients and showed highly significant difference at $P \leq 0.001$ on contents of phosphorus, calcium, magnesium, and iron. There was a significant difference at $P \leq 0.05$ on nutrients contents of sulfur, boron, and zinc. A quadratic relationship was found in response to pH treatments on phosphorus, zinc, and iron while a linear relationship was found in calcium, magnesium, and sulfur (Table 16).

The nutrients analysis showed higher values of potassium, calcium, magnesium, sulfur iron and manganese at low pH within pH treatments. However, the higher values of phosphorus and zinc were found at high pH.

Data from fruit peel's nutrients concentration show no significant difference in the effect of the interaction between rootstock and pH on macro or micronutrients within all rootstock and pH treatments. (Table 16).

Table 16.Effect of soil pH on fruit peel nutrients concentration of eight apple rootstocks in 2018 at Ithaca, NY.

| Rootstock | Soil pH | P (µg/g) | K (µg/g) | Ca (µg/g) | Mg (µg/g) | S (µg/g) | B (µg/g) | Zn (µg/g) | Fe (µg/g) | Mn (µg/g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | | | | |
| B9 | . | 838ab [Z] | 923abc | 372a | 640bc | 490a | 20.3d | 3.30a | 0.027a | 8.45ab |
| G11 | . | 760bc | 837c | 311b | 700a | 520a | 27.1c | 2.96a | 0.021a | 8.83ab |
| G202 | . | 909a | 953ab | 324ab | 640bc | 510a | 34.5a | 2.97a | 0.020a | 7.71b |
| G214 | . | 683c | 862bc | 340ab | 581c | 540a | 31.7ab | 3.12a | 0.024a | 8.60ab |
| G41 | . | 832ab | 928bc | 342ab | 663ab | 560a | 30.5bc | 3.46a | 0.027a | 9.31a |
| G935 | . | 866a | 883bc | 373a | 631bc | 510a | 33.3ab | 3.16a | 0.033a | 8.92ab |
| M26 | . | 804ab | 869bc | 312b | 670ab | 530a | 22.9d | 2.86a | 0.026a | 9.51a |
| M9 | . | 838ab | 1007a | 343ab | 690ab | 520a | 23.5d | 2.88a | 0.025a | 9.38a |
| Rootstock significance | | * | ** | * | * | NS | *** | NS | NS | NS |
| - | Low | 740b | 912a | 372a | 720a | 579a | 27.6a | 3.05ab | 0.027a | 10.3a |
| - | medium | 759b | 904a | 345a | 640b | 529b | 28.7ab | 3.28a | 0.026a | 9.50a |
| - | high | 954a | 890a | 306b | 590c | 467c | 25.5b | 2.81b | 0.024a | 6.97b |
| pH significance | | *** | NS | *** | *** | ** | ** | * | NS | *** |
| Regression | | Q*** | NS | L** | L** | L*** | NS | Q* | NS | Q* |
| Interaction means | | | | | | | | | | |
| B9 | Low | 774 | 942 | 420 | 610 | 500 | 21.7 | 3.22 | 0.037 | 8.98 |
| | medium | 810 | 919 | 390 | 630 | 50 | 19.3 | 3.39 | 0.023 | 9.13 |
| | high | 964 | 902 | 300 | 700 | 460 | 19.8 | 3.24 | 0.021 | 6.49 |
| G11 | Low | 665 | 821 | 307 | 580 | 570 | 28.8 | 2.82 | 0.020 | 9.42 |
| | medium | 741 | 815 | 333 | 750 | 510 | 27.6 | 3.28 | 0.023 | 9.44 |
| | high | 941 | 925 | 251 | 750 | 520 | 23.2 | 2.16 | 0.017 | 6.42 |
| G202 | Low | 882 | 972 | 385 | 540 | 590 | 39.2 | 3.33 | 0.018 | 9.07 |
| | medium | 818 | 952 | 347 | 600 | 500 | 36.8 | 3.41 | 0.020 | 8.47 |
| | high | 1010 | 946 | 278 | 730 | 480 | 29.4 | 2.22 | 0.020 | 6.08 |
| G214 | Low | 672 | 847 | 346 | 540 | 550 | 32.1 | 2.97 | 0.026 | 8.70 |
| | medium | 666 | 884 | 351 | 580 | 550 | 31.4 | 3.45 | 0.022 | 8.57 |
| | high | 759 | 849 | 292 | 770 | 430 | 31.5 | 2.61 | 0.020 | 8.28 |
| G41 | Low | 780 | 875 | 359 | 610 | 620 | 26.9 | 3.52 | 0.032 | 11.37 |
| | medium | 803 | 931 | 349 | 640 | 580 | 32.8 | 3.51 | 0.027 | 9.68 |
| | high | 981 | 951 | 302 | 790 | 490 | 24.7 | 3.23 | 0.027 | 6.58 |
| G935 | Low | 715 | 817 | 446 | 540 | 630 | 29.7 | 2.80 | 0.032 | 12.43 |
| | medium | 706 | 909 | 403 | 540 | 580 | 40.3 | 3.87 | 0.043 | 11.21 |
| | high | 996 | 888 | 335 | 690 | 450 | 31.3 | 2.95 | 0.027 | 7.09 |
| M26 | Low | 781 | 876 | 338 | 640 | 640 | 25.3 | 2.98 | 0.024 | 10.94 |
| | medium | 783 | 881 | 292 | 690 | 510 | 23.5 | 2.86 | 0.028 | 10.16 |
| | high | 856 | 846 | 313 | 68 | 470 | 19.6 | 2.72 | 0.027 | 7.19 |
| M9 | Low | 750 | 992 | 416 | 660 | 580 | 24.3 | 3.13 | 0.027 | 12.90 |
| | medium | 720 | 995 | 335 | 610 | 520 | 23.7 | 2.66 | 0.026 | 9.02 |
| | high | 1034 | 1030 | 303 | 790 | 470 | 22.8 | 2.92 | 0.023 | 7.38 |
| Interaction significance | | NS | NS | NS | NS | NS | NS | NS | NS | NS |

[Z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non significant effect, respectively.

## 4 Discussion

Rootstock Evolution.

As significant as it has been reported in several studies that rootstock evaluating is an important procedure to ensure characteristic stability and optimum field performance. Field evaluation faces lots of variable due to the field's soil heterogeneity. However, this experiment with the pot-in-pot set was designed to eliminate the field's soil variability. It was also aimed to assess the adaptation of eight rootstocks varying in their size category from dwarf to semi-dwarf under various soil pH conditions.

As mentioned in the literature review, Honeycrisp cultivar gained lots of market share due to its unique texture and balanced flavor which resulted in a high-value cultivar. However, the cost of maintaining healthy and sustainable production is high and required certain pre-planting strategies including chosen the proper rootstock. Since different rootstocks promote nutrient translocation of leaves and fruit of the grafted scion differently, the level of rootstock's efficacy in up-taking nutrients can be evaluated. However, soil pH is a critical issue within apple-growing area where nutrient availability becoming limited or in toxic level.

Very little was found in the literature on answering the question of which apple rootstock will perform better at different soil pH and can improve or sustain the best fruit quality of Honeycrisp. In this study, Honeycrisp was chosen as a cultivar model to test the effect of the rootstock interaction with soil pH and assess fruit quality and nutrient uptake and partitioning within the tree. In this set up a pre-planting soil pH was adjusted to mimic extreme field soil pH that allows apple to grow and to produce good quality fruit.

Nursery tree performance:

Before planting, all grafted rootstocks were grown and maintained in the nursery for two seasons to ensure a well-established tree with adequate side branches (feathers). This resembles a similar tree's size and age of what growers are getting their trees from commercial nurseries and allowing trees to produce fruits in potted under soil adjusted pH. In this study, trees were planned to follow the tall spindle training system with highly feathered and minimum pruning (Hoying et al., n.d.). The application of Maxcel with the Knip cut improved side branching production which agrees with findings reported by (Miranda Sazo and Robinson, 2012). The two-season period was excellent and produce a healthy tree with uniform growth and side branches that were set as base parameters measurement to evaluate changes over time and response to rootstocks and soil pH. Since the nursery's soil was the sandy, the uprooting tree didn't damage trees or its roots. Pre-planting screening of those trees also provides another level of maintaining uniformity within the same rootstock.

Adjusting soil pH.

The soil source for this experiment was selected properly followed by laboratory assessments and testing for pH malleability. The application rates for raising and lowering the soil pH was also tested in vitro using the buffer pH methods as described by (Cheng, 2015) while considering soil properties (particle size, oxidation rate) and soil conditions (original pH, buffering capacity, minerals present). Fine powder elemental sulfur (S) was used to lower soil pH in this experiment, however iron sulfate ($FeSO_4$), ammonium ($NH^{4+}$) based fertilizers and aluminum sulfate ($Al_2(SO_4)_3$) can be used to lower pH, but it was not recommended since it add-up nutrients that may

change nutrients availability level in lower pH treatments compared with higher pH treatments that will not receive additional nutrients. A monthly procedure of field soil's pH testing was conducted to check for soil pHBC and pH stability.

However, lowering soil pH was not progressing as predicted or at the same time period in a laboratory setup. This was noted in the low pH and some pots of medium pH where soil pH didn't show a decline from the original soil pH of 7.3. A frequent additional application of sulfur was done but uniform incorporating was not achieved since it caused damage to the roots. This was also understood that elemental sulfur needs to be oxidized by microbes to produce sulfate ($SO_4^{2-}$) and $H^+$, leading to lowering the soil pH. This agrees with elemental sulfur usage to lower soil pH previously described by (McCauley et al., 2009). Thus, a stable pH was noted in the second growing season and no additional applications were needed in medium pH treatments while few pots on low pH treatment required additional sulfur.

In the higher soil high pH, a pH decline was noted in many pots and required additional $CaCo_3$ (CaLCarb). This might be due to nitrogen fertilization that leads to acidifying the soil as reported by (Tian and Niu, 2015) finding that nitrogen deposition induced soil acidification.

Pot-in-pot set up

Although the pot-in-pot setup has been successfully tested in several experiments and was reported in many publications, placing pots in trenches was complicated, laborious and tedious setup. The field conditions were also not accessible by machinery during the initial establishment stages which required manual labor. Moreover, the trenches soil was not settled and caused pots to get a lower level than the

original set level and leading to be backfilled with field soil during raining in a few pots. Trees were grown in 15-gallon pots under limited soil fertility level and subjected to varying soil pH. Due to decreasing root zone volume in pots, tree's growth was also limited to those factors and thus flowering and flower sets were affected by these stress conditions. This study supports evidence from previous observations on planting on pots system (Bar-Yosef et al., 1988; Carmi, 1986) which reported that smaller pots volume will result in decreased yield, total dry matter production, and N and water uptake rates.

Soil nutrients analysis

Due to unstable pH during the first growing the nutrients analysis was not done however, only field soil pH testing was conducted. Average soil nutrients from each soil pH level were reported in tables 6 and 7 in the result section but no soil nutrients comparison was done between the growing seasons. Soil analysis revealed that at pH 6.5 treatment, optimal levels of P (5.29 ppm) and K (29.38 ppm) and Ca (3.93ppm) and Mg (27.33 ppm) in both growing seasons. This finding is consistent with that of (Fazio et al., 2018b).

Field growth performance.

In the first growing season in 2017, no significant difference was noted in tree height within the eight tested rootstocks and that was predicted since the change in soil pH was not effective or detectable. However, soil pH showed to affect tree height when comparing changes of all rootstocks within pH treatments. Statistical data showed that all rootstock perform better at medium pH and high than rootstocks grown at lower soil low pH treatments in the first growing year.

However, the trunk cross-section area TCSA showed a highly significant

difference within rootstock and that due to different rootstocks size groups. The higher increase in TCSA was found in rootstocks G.202 and M.26 indicating better growth. While no significant effects of soil pH were noted on tree's TCSA in the first growing season.

During the second growing season in 2018, a highly significant difference was found in the tree's height within rootstocks. The changes in the tree's height were found higher in rootstocks G.935 and G.41 and the low in rootstocks B.9 and G.214. However, no effect of soil pH on the tree's height was found in the second year compared to the first growing year.

Also, a highly significant difference was found on the TCSA within rootstocks in the second growing year. Rootstocks G.935 and M.26 had a higher increase of TCSA than all other tested rootstocks. Soil pH treatment has a significant effect on TCSA in the second year and all rootstock found to have a higher increase in TCSA at high pH treatment that other soil pH treatments. The interaction between soil pH treatments and rootstocks showed no significant difference in all tested growth parameters (tree's height and TCSA) in both growing seasons suggesting that solo effect of the rootstocks or pH are more detectable than combined effect. This also accords with earlier finding which showed that the rootstocks, plant growth was affected adversely by low or high pH treatments (Fazio et al., 2012). However, no single soil pH treatments were optimum growth for all rootstocks. However, when tree's growth was examined in different rootstocks, each rootstock exhibited a different pattern of growth as influenced by soil pH which was also noted by Fazio (Fazio et al., 2012).

Carbon and nitrogen

The main hypotheses of this study were that soil pH will affect nutrients availability and thus affecting nutrients uptake and assimilation. Also, based on previous results, apple rootstocks exhibit different uptake level. In this study, a significant difference of N, C leaf content was found within the tested eight rootstocks in both growing seasons. However, Rootstock G.214 found to acquire higher C and N in the first year while B.9 had higher content in the second year (Saiedyfar and Asgari, 2014).

The soil pH treatment was only affecting leaf carbon percentage during the first growing season in 2017 while no difference was detected within pH in nitrogen and carbon in the first year. The same was noted in the effect of the interaction between rootstock and pH treatments, where an only significant difference was only noted in the leaf carbon in the first growing season. However, when carbon and nitrogen were inspected by rootstocks, each rootstock exhibited a different pattern of carbon and nitrogen ratio influenced by soil pH.

Leaf macro and micronutrients analysis

All macro and micronutrients contents except for B and Fe showed a significant difference in the first year within rootstocks. However, no single rootstock was found to have better nutrients uptake of all nutrients. All rootstock had higher absorption level of P, S, B, and Fe when grown under low pH and higher K, Ca, Mg, Zn and Mn under high pH which agrees with other findings (reference). The interaction between rootstocks and pH was also found significant in all macro and micronutrients except for B, Fe, and Mn. This finding was also reported by (Aras et al., 2018) where they reported that a reduction in pH contributes to the transformation of some elements like Fe from

indissoluble form to soluble in high lime content and higher pH in soils.

In the second year growing season, the main effect of rootstocks showed also a significant effect on all nutrients except for S, Fe, and Mn. This could be due to continuous fertigation with NPK and iron along with additional applications of sulfur to adjust the soil pH. However, when each nutrient was inspected individually by rootstocks, each rootstock exhibited a different pattern of absorption influenced by soil pH. This finding was also reported by (Neilsen and Hampson, 2014) is consistent with the finding in our study regarding the difference among rootstocks with superior and inferior abilities to accumulate individual nutrients, but only in rootstocks G.935 for (Ca, Mg, S, Fe, and Mn) and G.202 for (K and B) were superior for more than a single nutrient in the second growing season in 2018. The ability to accumulate a range of key plant nutrients was not correlated with tree height increase or TCSA.

Many factors contribute to the complexity of studying leaf and fruit mineral concentration. One of the most significant factors is that individual observation of both fruit and leaf mineral concentration varies considerably. This variability in concentration is related to many factors including nutrient availability, crop load, and environmental condition (Faust, 1989). Additionally, a major difference in nutrients concentration was reported among years and within a season (Neilsen and Neilsen, 2003).

Fruit's maturity parameters

In this study, all rootstocks produced less fruit per tree than the average Honeycrisp production in the second growing season due to many factors. Trees were grown in 15-gallon pots under limited soil fertility level and subjected to varying soil

pH. Due to by decreasing root zone volume in pots, tree's growth was also limited to those factors and thus flowering and flower sets were affected by these stress conditions. This study supports evidence from previous observations on planting on pots system. However, the average yield was ranging from 3-10.3 fruits per tree with maximum yield was produced by trees on rootstock G.41 under low pH and high pH while trees on rootstock B.9 produced similar yield an average of 10.1 fruit per tree under medium pH. The lowest yield per tree was found on rootstock G.202 at low pH, and in rootstocks G.202 at high pH with 3 and 3.3 fruit per tree respectively.

These results corroborate the findings of a great deal of the previous work about increased bulk density in pots caused significant reduction in shoot growth with varying degree of effect among rootstocks (Ferree et al., 2004).

Fruit weight and skin color were affected by different rootstocks. Heavier fruits were found on trees from rootstock M.26 and G.11 gaining an average of 212 and 211grams per fruit. While G.41produces smaller fruit on average of 158 gram due to higher yield.

This study has been unable to demonstrate an overall correlation between fruit size, fruit weight, TSS, firmness yield and bitter pit incident with soil pH with finding reported by an earlier study (Serra et al., 2016). However, a negative correlation was found between yield and TSS only in B.9 rootstock which confirms previously finding on different rootstocks have different growth patterns leading to different fruiting quality and interactions (Fazio et al., 2012).

Since the soil pH has a significant effect on fruit maturity parameters, it was worth running a correlation to investigate the effect on the fruit qualities at each

rootstock individually. While soil pH positively correlated with fruit weight and size on rootstocks B.9 and G.41, no effect of soil pH was found on other tested rootstocks in this study. Soil pH treatment was found to have a negative correlation with fruit's firmness in rootstocks B.9 and M.9 but not shown in other rootstocks. However, a negative correlation was shown in rootstocks M.9, M.26 and G.202 between soil pH and total soluble solids (TSS).

In rootstock M.9, soil pH was found to have a negative correlation with the yield. Other correlations were also noted individually within fruit's parameters such between fruit's weight and fruit' red skin percentage and between yield and fruit size, however, that correlation was not significant to the main effect of soil pH or rootstocks.

Fruit storage disorder

Due to a low yield per tree, this study could not conclude that the effect of the rootstocks or soil pH were indicators on the fruit storage disorder incident of the bitter pit or soggy breakdown. Baugher reported that the percentage of fruit developing bitter pit on Honeycrisp varied depending on year, orchard, and tree within an orchard (Baugher et al., 2017). However a correlation was found between bitter pit incident percentage and nutrient ratio in Mg/Ca, P/Ca, K+Mg/Ca and K+Mg+P/Ca within all rootstocks. A future comparative yield from several fruiting seasons will present better resolution data that can be used as a physiological disorder predictor.

Fruit peel nutrients

Fruit mineral analysis provides a great tool in assisting growers in understanding nutrient requirements in the orchard as well as providing a predictive indicator for the susceptibility to physiological disorder (Baugher et al., 2017). Statistical data showed

that pH treatments highly affecting peel nutrients such as P, Ca, Mg, and B while no effect was found on K, S, Zn, Fe, and Mn.

Multivariate correlation analysis indicated bitter pit was very correlated to the fruit peel ratios of P/Ca, Mg/Ca, (K + Mg)/ Ca, and (K+ Mg+P)/Ca) which agreed with finding reported by Baugher (Baugher et al., 2017). It also showed a correlation between soil pH treatments and ratios of P/Ca, Mg/Ca, (K + Mg)/ Ca, and (K+ Mg+P)/Ca) (Table 18).

It has been suggested by (Cheng, 2016; Shoffe et al., 2014) that Honeycrisp' fruit has lower peel Ca levels than other cultivars which associated with the lowest bitter pit levels in 'Honeycrisp'. This does not appear to be the case in this study where no correlation was found between fruit peel nutrients and bitter pit incidents due to low yield per fruit.

A study by (Fazio et al., 2018b) on testing the effect of the rootstock on fruit nutrients concentration found that Honeycrisp fruit from B.9 showed the lowest overall values, while G.210 and G.41 had the highest for all nutrients tested. Our outcome was contrary to those findings were no rootstock acquired a higher level of all nutrients with no patterns in nutrient values were noted among rootstocks.

However, there are similarities between the fruit nutrient concentration expressed by different rootstocks in this study and those described by (Neilsen and Hampson, 2014).

Phosphorous, magnesium, Sulfur, Mg/Ca, P/Ca, K+Mg/Ca and K+Mg+P/Ca values were positively correlated with pH treatments, among all rootstocks (Table 16). Several associations vary between positive and negative associations were found between values of peel nutrients and the fruit parameters such as weight size and red color skin percentage.

## Correlations

Table 17.Correlation of fruit peel nutrients concentration of potassium, Magnesium and sulfur from 'Honeycrisp' grown on 8 rootstocks under varying soil pH levels at Ithaca, NY.

| | pH | P | Mg | S |
|---|---|---|---|---|
| pH | 1.0000 | 0.4688 | 0.4464 | -0.4152 |
| P | 0.4688 | 1.0000 | 0.6049 | 0.0027 |
| Mg | 0.4464 | 0.6049 | 1.0000 | -0.3094 |
| S | -0.4152 | 0.0027 | -0.3094 | 1.0000 |

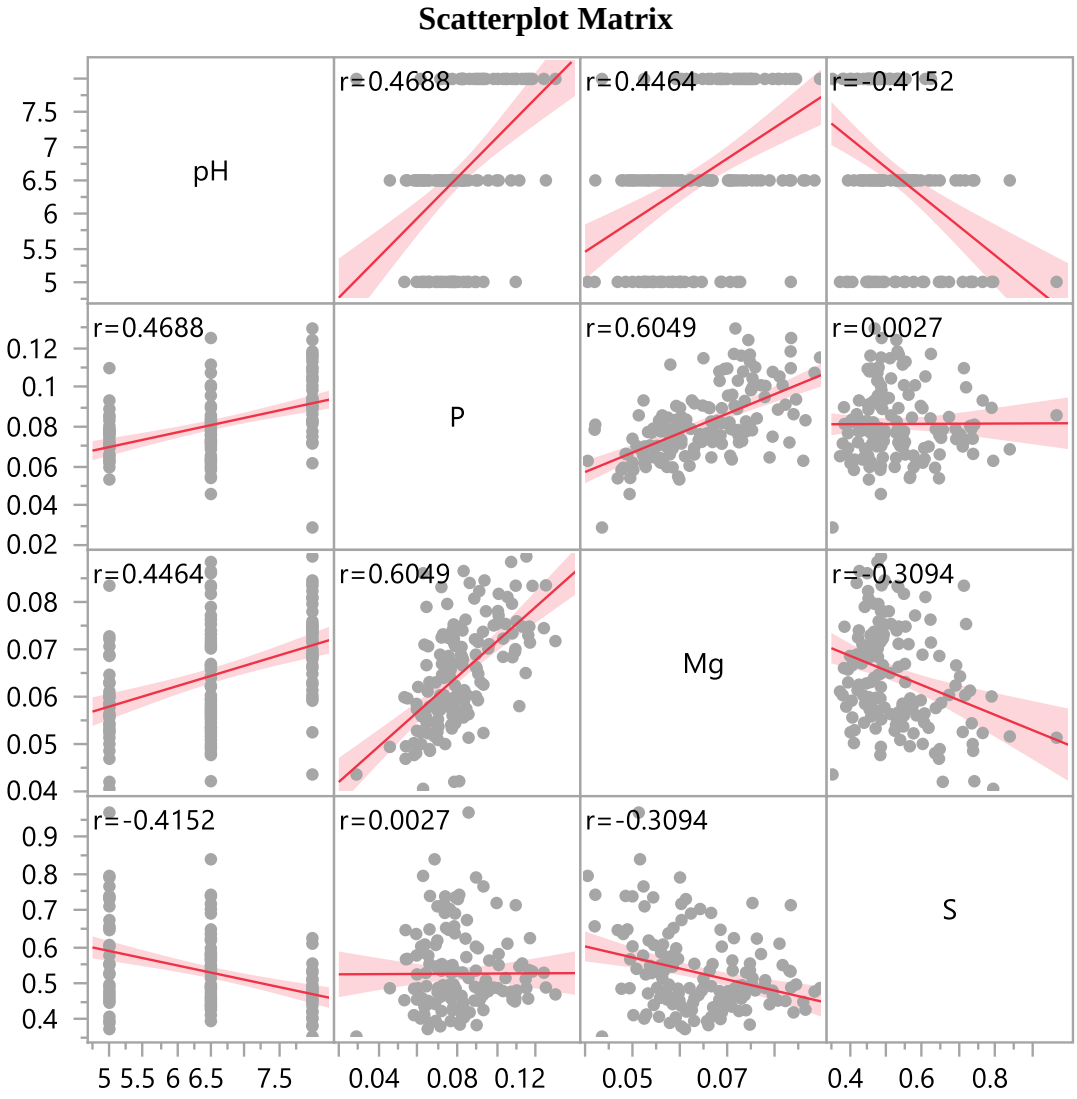


Figure 10.A multivariate scatterplot of means of fruit peel nutrients concentration of potassium, magnesium and sulfur from 'Honeycrisp' grown on 8 rootstocks under varying soil pH levels at Ithaca, NY.

## Correlations

Table 18.Correlation of bitter pit incident with fruit peel nutrients ratios from 'Honeycrisp' grown on 8 rootstocks under varying soil pH levels at Ithaca, NY.

| | pH | bitterpit | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2225 | 0.4673 | 0.5129 | 0.4702 | 0.5003 |
| bitterpit | 0.2225 | 1.0000 | 0.5180 | 0.4384 | 0.5095 | 0.4215 |
| Mg/Ca | 0.4673 | 0.5180 | 1.0000 | 0.8538 | 0.9831 | 0.8197 |
| P/Ca | 0.5129 | 0.4384 | 0.8538 | 1.0000 | 0.8870 | 0.9916 |
| K+Mg/Ca | 0.4702 | 0.5095 | 0.9831 | 0.8870 | 1.0000 | 0.8759 |
| K+Mg+P/Ca | 0.5003 | 0.4215 | 0.8197 | 0.9916 | 0.8759 | 1.0000 |

## Scatterplot Matrix

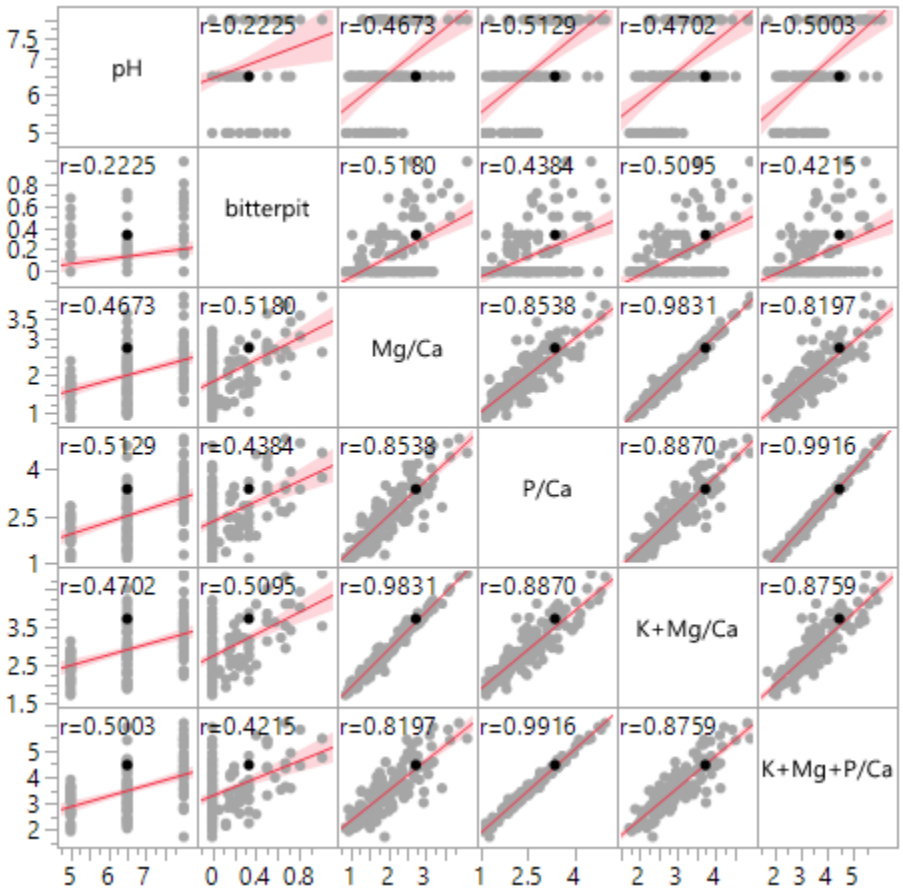


Figure 11A multivariate scatterplot of means of fruit peel nutrients elements and nutrients ratios and fruit weight from 'Honeycrisp' grown on 8 rootstocks under varying soil pH levels at Ithaca, NY

# 5 Conclusion

The present study was designed to determine the effect of soil pH on the performance of eight apple rootstocks in terms of vegetative growth, leaf nutrients uptake and fruit quality and nutrients composition.

The results of this study confirmed the effect of soil pH on the availability of soil nutrients and that different rootstock responded differently to varying soil pH due to a different response to nutrient availability and deficiency. The rootstock vegetative growth, nutrients uptake, and fruit quality were also affected by both; rootstock and soil pH suggesting an interaction effect of both main effects.

Statistical data showed that all rootstock growth performance was better at medium and high pH than at lower soil pH treatments. These findings suggest that in general soil micronutrient deficiency at low pH had no direct effect on tree growth and that some apple rootstock thrives at high pH under this experiment conditions.

A correlation was found between fruit quality and soil pH and between fruit quality and nutrients availability. However, different correlations (positive or negative) were found at rootstock level. Taken together, these results suggest that suggesting this is due to soil pH and to different nutrient uptake capacity of each rootstock.

The more correlated pH treatment to leaf and fruit nutrient contents, the more tight correlation to the effect on the tree's growth and fruit quality. The findings of this investigation complement those of earlier studies regarding the abundance of nutrients available for plants found at soil pH ranging from 6.0 to 7.0.

Although one limitation of this study is the yield of the second growing season was less than the average fruit per tree of Honeycrisp cultivar, results of this research

support the idea that a there are correlations between bitter pit incident percentage and nutrient ratio in Mg/Ca, P/Ca, K+Mg/Ca and K+Mg+P/Ca.

The insights gained from this study may be of assistance to both apple rootstock breeder to identified better rootstock performance at varying pH and fertility level and to apple growers with extreme soil pH orchards. It also establishes a quantitative framework for detecting further phenotypic characteristics of apple rootstock that can be linked to genotypic traits.

Although this study addresses the effect of the soil pH on one fruiting season, multiple yield comparison will provide higher resolution data that can recommend rootstocks based on field soil pH and nutrients balance.

This study could be further improved to provide an assessment of other apple cultivars that might be sensitive to soil pH and consequently to soil nutrients dynamics. It also could be improved by adopting more leveled and more machinery accessible orchard to facilitate horticultural practice and minimize variability due to field condition and drainage.

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# CHAPTER THREE

## Optimizing Aeroponics Systems for Assessing The Architecture And Dynamics Of Root Distribution Of Four Apple Rootstocks In Response To Varying Solution pH

*Abstract*

Nutrients solution's pH is very important growing medium parameters that determine the availability and mobility of nutrients for plant uptake. Understanding the root's distribution and architecture of apple rootstock would provide a precise recommendation for rootstock selection. Thus, monitoring apple root's dynamics in adjusted soil pH would increase the knowledge of how root distribution is responding to a range of pH. To date, few quantitative tools have been implemented to measure this interaction and deliver critical quantitative experimental data on apple rootstocks.

This study aimed to perform real-time monitoring of the root system using optimized aeroponics systems that were designed and made at Cornell AgriTech Geneva, USA.

Four Geneva® apple rootstocks (G.210, G.214, G.41, G.890) were tested in the aeroponics system under nutrient misting.The nutrients' solution's pH was adjusted to a range of pH values (5.5, 6.5 and 8.0). Roots were monitored during 30 days of growth and evaluated regularly based on distribution and root mass. Images of developed root grown in the aeroponics system were taken at the end of the experiment and analyzed using GiaRoot® software.

The statistical results from the aeroponics study showed no difference of root's architecture parameters within the four Geneva rootstocks. However, when each rootstock was investigated individually, rootstock G.210 found to have a higher root

width diameter, network width and network bushiness which reflected in their higher root network volume and network convex area. While rootstock G.214 showed higher number of connected root component which leads to higher network surface area and higher length distribution.

Solution pH was found to affect significantly all root parameter measurements. Under solution pH 5.5, the root width diameter was higher among pH treatments. However, all other parameters, number of connected components, maximum number of roots, network depth, and network width and network bushiness, showed doubled or tripled improvement at pH 8.0. Statistical data from root nutrients analysis showed no significant difference in P, K, Ca, Mg, S, B, Zn, Cu, and Fe within tested rootstock grown in an aeroponics system.

## 1. Introduction.

The root system and its architecture have a vital role in supporting the overall tree growth and developments. Besides the basic role in supporting tree's anchorage and storage, the root architecture and distribution are responsible for navigating the soil profile and sensing moister and nutrients. Understanding the aboveground growth of plants should be accompanied by understanding the belowground growth to assess the interaction which drives the overall growth. It is true for apple trees and more precisely for grafted trees because it is a combination of two different genotypes.

Evaluation of Geneva apple rootstocks populations has shown that there is plentiful genetic variation leading to different root architecture traits (Fazio et al., 2015). A remarkable feature was reported in some selected Geneva rootstocks presented as the "fine root" trait, described as an increased branching of the root system. This abundance of fine roots eventually increases the root surface area and improve the exploration capacity of the soil volume by the roots (Fazio et al., 2015). Many theories have been advanced about the possibility that root traits may influence the overall tree growth and productivity by modifying nutrition and root/shoot partitioning ratios.

Fine roots are considered to be the most active and dynamic part of the root system. They play an important role in scouting and navigating below ground soil for water and nutrients as well as in nutrients uptake (Artacho and Bonomelli, 2016). It is well understood that the formation of lateral roots increases the sink strength of the root system and encourages the development of greater root length. This ultimately leads to better nutrients and water acquisition.

The large majority of published studies have been dedicated to understanding the

aboveground parts of the apple trees, however, root behaviors and developments have not been fully explored. This was not only due to the complexity of the rhizosphere but due to limited availability of non-destructive techniques that were optimized for fruit trees in general and apple trees specifically.

Studies found that apple root systems have distinctive seasonal growth patterns which influence their nutrients and water exploration, along with the formation of symbiosis colonization with mycorrhizae (Eissenstat et al., 2006). These traits could be associated with genes, gene expression patterns, and physiological attributes. Since most of the studies are only providing information about the scion's traits; more research is needed to understand the roots traits to facilitate faster selection based on genetic markers. (Fazio et al., 2015).

### 1.1. Purpose and Significance of the Study,

Several studies have tested and confirmed field performance of new apple rootstocks (Autio et al., 2008; Autio et al., 1996; Reig et al., 2018; Russo, Robinson, Fazio, & Aldwinckle, 2007; Schupp, 1995). However, the root architecture of theses rootstocks has not yet been under investigation. Investigating the hidden parts of the apple rootstock will provide significant information on rootstock behavior and fine root distributions leading to better water and nutrient acquisition.

It has been challenging to research various interactions between plant and ecosystem due to the magnitude of diversity and heterogeneity in how plant species are acquiring resources, storing and turning them over (McCormack et al., 2017). The deeper knowledge about the differences in plant's responses to rhizosphere's dynamics can yield measurable changes in plant traits including anatomical, morphological, chemical and

physiological phenotypes (McCormack et al., 2017). Until now, most of the studies on connecting plant traits to the ecosystem is limited to above ground parts. Only a few researchers were focusing on the below our feet to gather more informative interaction (Jégou et al., 2001). Publications that concentrate on apple roots more frequently adopt destructive methods or non-dynamic approach. As those approaches have been providing valuable information in regard to root mass and chemical composition, still were limited to certain stages of growth.

Understanding the form and availability of resource pools for apple trees and the dynamic flux rate is very important. This information is essential to determine the interaction between the whole plant-system and the rhizosphere. Moreover, knowing what apple rootstock contribute to rhizosphere productivity and cycling resources provide a significant knowledge of fluxes impact in a given soil condition.

Genotypic variation explains how apple roots may retain different growth patterns and nutrient uptake efficiencies (Fazio et al., 2015). These root's configurations are also influenced by other external factors such as soil type and soil pH which ultimately affect the scion nutrient status.

Plants require water, mineral nutrients, light, and carbon dioxide in order to sustain growth and produce fruit. Water is delivered through the substrate, and many macro and micronutrients are it dissolved in it. However, the mobility of these nutrients is controlled by pH (Pennisi and Thomas, 2005). Macronutrient, nutrients required by the plant in large quantities, are largely available for plant uptake in a pH range of 6.2-7.3. Especially affected by pH are micronutrients or trace elements which are nutrients required in small quantities. When the pH is low, strongly acid soil, the mobility of

micronutrients is generally increased. Thus, the plant could absorb them in excess of what needed, eventually leading to toxicities. On the other hand, when the pH is high (strongly alkaline soil), the micronutrient's mobility is drastically reduced, the plant cannot absorb enough, resulting in nutrient deficiencies.

In addition to quantifying the response of root architecture and dynamics the optimization of aeroponics systems to study apple rootstock was one of the objectives of this study.

This information would also help apple rootstock breeders select elite pedigree for their hybrids. It also would provide precise recommendations for the grower to select proper rootstocks to match their soil properties and fertility level.

### 1.2. Theoretical Basis for this Study

To monitor the activities of rootstock's roots under a range of soil pH, a special setup was devised to measure the changes in growth parameters in response to nutrients availability. The measurement of root behavior and dynamics requires frequent monitoring and repeated sampling and observations in a controlled environment. These conditions could be achieved in a soilless medium or under rhizotron setup.

The limited availability for such systems oriented toward studying fruit trees is a constraint to study apple root dynamics and architecture. Hence the need to optimize a system to accommodate growing apple trees in an environment with the ability to collect repeated data and growth parameters.

Aeroponics growing systems have been used in many studies to investigate the root development in many plant species but was limited to small plant and shrubs (AlShrouf, 2017). This system allows growing apple rootstock in regulated misting

nutrients while roots are suspended inside a growing tank. Root growth and distribution are driven by their genetic and hormonal influence and not restricted by soil boundaries. It also enables repetitive non-destructive observations and sampling. By analyzing periodical images of the root system under each pH level, a clear explanation can be drawn by measuring root growth parameters such as; root surface area, fine root diameter, branching, total convex area and root network area.

### 1.3. Justification and hypothesis.

New scientific tools can provide clear quantitative data representing root parameters in vivo. Such tools could also distinguish between the root and shoot specific effects as well as quantifying the influence of morphology and physiological functions on nutrient transport and uptake. Understanding the development of apple rootstock roots and their spatial distribution and understanding the relationship between root and shoot development subsequently can be of great significance for the apple and temperate fruit industries. The goal of this study is to optimize a methodology to monitor the response of apple's rootstocks grown in vivo in a range of solution's pH with repeated and accurate sampling and measurements.

Preliminary work conducted in 2014-2015 using the aeroponics growing system showed that an apple rootstock breeding population segregates for different root architectures. This is a follow up study which sought to optimize a methodology to assess root's developments grown in modified aeroponics for commercial apple rootstock. The experiment was conducted to compare four Geneva rootstocks, G.41, G.210, G.214 and G.890 under three levels of pH solution.

### 1.4. Objectives:

Direct observation and nondestructive measurement of root growth and developments have been always a challenge under field conditions. Moreover, because of the limited availability of information on the growth of apple rootstock, this study sought to: 1. Optimizing an aeroponics system to serve as a scientific tool to study apple rootstocks in varying solution's pH. 2. Monitor and correlate fine root production and turnover rate in response to differences in solution pH. 3.Compare rootstocks' root architecture of new root production in aeroponics by utilizing images processed by root image software. 4.Assess the nutrient uptake efficacy under pH treatments. 5. Monitor root development in time-lapse photography to understand factor influencing root growth and development.

Measurable observation of a plant's root system in a non-destructive way can provide more accurate data with regards to root growth and development. Experimentation of new methods that facilitate in-vivo monitoring in reproducible controlled growing system was an essential component of this study. . This allowed repetitive sampling and observation without disturbing the growth flow and normal distribution.

## 2. Materials & Methods:

### 2.1. Aeroponics design and setup

**Aeroponics tanks**

High-density polyethylene (HDPE) black tanks were used in this experiment. Nine tanks were optimized to serve as a nutrient solution tank and to hold plants. The dimensions of each tank were 60cmx60cmx 50cm (length x width x height). Tanks were equipped with a top cover that house 9 square shape openings with square lids. In the center of each lid, a foam collar insert was placed to hold the plant in an upright position and the lower part suspended. The spacing between the centers of the collar insert to the adjacent was 20cm.

Tanks were equipped with inner spraying rails positioned 25cm under the top cover with 14 misting nozzles to provide a fine mist of nutrients and oxygen mixture to the root zone. The spraying rail was designed in a rectangular shape to ensure complete coverage of nutrients mist surrounding the plant's root. Ten Side misting nozzles were a 360° type while the four corner nozzles were 180°. Side inlet and bottom drainage fitting were installed in each tank to maintain nutrients circulation. Tanks were positioned in a mobile platform to facilitate movements and accessibility.

**Nutrients reservoir.**

Polyethylene resin tanks were used to hold the nutrients solution. The dimensions of the tanks were 30cm x 30cm x 90cm (length x width x height). Two fittings were installed in the nutrients reservoir to serve as feed and return lines at 40cm and 60cm height respectively.

**Stock nutrient solution.**

Solution nutrients were prepared following the recommended concentration by the manufacturer of 1g per 3.8 liters of water using Jack's nutrients fertilizers of 5-12-26. (JR PETERS Inc. USA). The solution is prepared by mixing the nutrients fertilizers with warm reverse osmosis (RO) water then the solution's pH was adjusted to 5.0, 6.5 and 8.0. The acid formulation uses food grade phosphoric acid was used to lower the pH where base formulation using potassium hydroxide and potassium carbonate was used to raise the pH to the required level. The volume of the nutrient solution in each nutrient reservoir was maintained at 90 liters throughout the experiment. The nutrient's solution was drained, and the system was flushed, filters were replaced, and fresh nutrient's solution was added every week.

**Pumps.**

The adopted system was installed based on low-pressure aeroponics system that was designed and made at Cornell AgriTech, Geneva USA. Two pumping systems were installed in the aeroponics system. A ½ horsepower rotary positive displacement single stage mechanical pumps were used to supplies nutrients from the nutrient's reservoir to aeroponics tanks. Another 1/8 horsepower magnetic drive pumps were used to drain the aeroponic tanks and return nutrients solution to the nutrient's reservoir (Littlegiant. Franklin Electric Co., Inc. USA). The nutrients solution was filtered twice, before passing through the pump and before entering the reservoir tank to prevent particle blockage within the system. To control the misting at the required time interval and circulating the nutrients, digital timers were linked to the pumps and the power supply.

The misting timers were set to spray for 10 seconds every 120 seconds. The circulating pump was set to drain the solution from tanks for 90 seconds every 8 minutes.

The aeroponic unit was kept in a greenhouse at temperature (27–30 °C) and relative humidity (60%) while temperature within mist chamber (rhizospheric zone) was 28–30 °C with 80–90% of relative humidity.

### 2.2. Experiment layout and treatment.

Three aeroponics tanks were connected using a flexible polyethylene hose to a single nutrient's reservoir where the solution's pH was monitored and regulated by a pH dosatronic (Bluelab® Corporation Limited. New Zealand). Three solution pH treatments were used pH 5.0, 6.5 and 8.0. Each treatment has three aeroponics tanks distributed in rows. Uniform apple rootstocks were selected and plugged into the designated aeroponics tanks. Each pH treatment was assigned three aeroponics tanks connected to one nutrient reservoir.

**Experimental design:**

A total of two hundred and sixteen plants were divided into three cycles. Each cycle consists of seventy-two plants of four rootstocks. The experimental layout was completely randomized and consisted of four combined rootstocks (G.890, G.210, G.41, and G.214) and three pH treatments (pH 5, 6.5 and 8). Each pH treatment was replicated thrice with two plants per replicate by using six trees of each rootstock (24 plants per treatment). Two plants of each rootstock were plugged randomly into each aeroponics tank totaling 8 plants per tank. Three replicates were used in this experiment with two plants of the same rootstock per replicates.

### 2.3. Plant materials:

The plant materials used in this experiment were a 1-year old tissue culture propagated rootstocks supplied by commercial nursery (North America Plants. Inc USA). Four commercially available apple rootstocks were selected to be investigated Geneva®

rootstock G.890, G.210, G.41, and G.214.

Prior to planting into the aeroponics system, plants were grown into potting mix soil for one year with regular nursery maintenance. A preliminary assay was conducted to screen and evaluate individual plants based on consistent height and stem diameter. When the stem's diameter was 5-6mm, plants were removed from the potting soil and washed and sprayed with fungicide. 50% of the lower roots were pruned using sterilized scissors to maintain uniform size and all old and yellow leaves were removed. Plants were left with all roots intact for 7 days to acclimate to the aeroponics system and then roots were reduced and 50% of the root volume was cut. After three days, all old roots were then removed from all plants by cutting the lower 1-2 cm shoot where old roots were forming. Two plants from the same rootstock were plugged in each tank.

**2.4. Data collection and sampling**

Regular pest and disease inspection and application were carried out during the four weeks of growth. 10 days after plugging, the plants were toped to a height of 35 cm. After 30 days, plants were removed from the aeroponics tanks and root's images were taken for further images analysis and comparison. Each plant was held in an upright position to take a high-resolution image of the one-dimension picture. All root images were taken from the exact position with consistency distance to maintain the aspect ratio. Pictures were taken using Canon EOS 50 D DSLR camera.

Roots were harvested by cutting the newly formed roots in aeroponics condition using sterilized scissors and placing them in liquid nitrogen while processing. Samples were then used for RNA extraction and nutrients analysis. A total destructive sample was prepared after 4 weeks growing period in the aeroponics.

**2.5. Image processing software.**

All photos were processed by GiA Roots (Georgia Tech Research Corporation and Duke University). GiA Roots stands for General Image Analysis of Roots. GiA Roots is a high throughput software tool to automate and facilitate the large-scale analysis of root system, architecture, and networks. GiA Roots has been designed to help scientists and breeders quantify the structure of plant root system architecture, regardless of their prior training in mathematics and computer science. This software was designed to handle a very large number of root photos by:

1. Performing the necessary data pre-processing to clean up noise that arose during the imaging step.
2. Calculate root system architecture features of individual images.
3. Export all the calculated features for downstream analysis, e.g., statistical analysis of the relationship between genotype, phenotype, and environment.

The following parameters were measured and generated by the software:

1. Average root width: the mean value of the root width estimation computed for all pixels of the medial axis of the entire root system.
2. Ellipse axis ratio: the ratio of the minor to the major axis of the best fitting ellipse.
3. Major ellipse axis: the length of the major axis of the best fitting ellipse to the network.
4. Maximum number of roots: after sorting the number of roots crossing a horizontal line from smallest to largest, the maximum number is the 84th-percentile value (one standard deviation).

5. Median number of roots: the result of a vertical line sweep in which the number of roots that crossed a horizontal line was estimated, and then the median of all values for the extent of the network was calculated.
6. Minor ellipse axis: the length of the minor axis of the best fitting ellipse to the network.
7. Network area: the number of network pixels in the image.
8. Network bushiness: the ratio of the maximum to the median number of roots.
9. Network convex area: the area of the convex hull that encompasses the image.
10. Network depth: the number of pixels in the vertical direction from the upper-most network pixel to the lower-most network pixel.
11. Network length: the total number of pixels in the network skeleton.
12. Network length distribution: the fraction of network pixels found in the lower 2=3 of the network. The lower 2=3 of the network is defined based on the network depth.
13. Network perimeter: the total number of pixels connected to a background pixel (using an 8-nearest neighbor neighborhood).
14. Network solidity: the total network area divided by the network convex area.
15. Network surface area: the sum of the local surface area at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image.
16. Network volume: the sum of the local volume at each pixel of the network skeleton, as approximated by a tubular shape whose radius is estimated from the image.

17. Network width: the number of pixels in the horizontal direction from the left-most network pixel to the right-most network pixel.
18. Network width to depth ratio: the value of the network width divided by the value of network depth.
19. Number of connected components: an integer denoting the number of connected groups of network pixels in the image after image pre-processing. For example, if all network pixels in the thresholded image are connected to all others via a contiguous path of nearest neighbor pixels then the value is 1. If the root network has a break in it somewhere that separates the network into two sub-networks, then the value is 2. Note that a real root should only have 1 connected component, but due to errors in image acquisition and pre-processing, the value may be greater than 1 and can be used as a quality control value.

## 3. Results

The aeroponics system was designed to accommodate a total of 72 trees in a single growing cycle, thus for this experiment, the design was duplicated into 3 cycles. In November 2018, the first set of 72 plants were plugged into the aeroponics to represent cycle one. After four weeks of growth, plants were removed from the system, root's growth was assessed and photographed, and roots were harvested for both nutrients' analysis and molecular assessments. (RNA extraction). The second cycle was plugged in December 2018 with identical rootstock and treatments and harvested in January 2019. The third and the final cycle was plugged in January 2019 and harvested in February 2019.

Dataset generated by GiaRoot® software were processed by SAS ®, Version (9.4). SAS Institute Inc., Cary, NC, 1989-2019 statistical. Data from three cycles were combined as a single dataset. Root's architecture's parameters were; Average Root Width, Network Bushiness, Ellipse Axes Ratio, Major Ellipse Axis, Maximum Number of Roots, Network Width, Minor Ellipse Axis, Network Area, Network Perimeter, Network Solidity, Network Surface Area, Network Length, Network Volume, and Network Width to Depth Ratio). Other parameters either skewed to the right (Network Length Distribution, Median Number of Roots, Network Convex Area and Specific Root Length) or skewed to the left (Network Depth).

### 3.1. Root growth

Only fourteen root parameters were selected for this statistical analysis due to the short growing period of 4 weeks only. Those root architectural parameters were grouped based on descriptive similarity. The first basic root group includes; root width diameter,

number of connected components, maximum number of roots, network depth, network width, and network bushiness.

The Average mean is the mean value of the root width estimation computed for all pixels of the medial axis of the entire root system. The network depth is the number of pixels in the vertical direction from the upper-most network pixel to the lower-most network pixel. While the network bushiness is the ratio of the maximum to the median number of roots. The number of connected components (NCON), an integer denoting the number of connected groups of network pixels in the image after image pre-processing.

The independent variables were the solution's pH and rootstocks. The statistical analysis showed that all rootstock produce similar root architectural dimensions in the first class with no significant difference four rootstocks. However, a highly significant difference at $P \leq 0.001$ was found in 'number of connected components', network depth, and network width and significant differences at $P \leq 0.005$ were found in root width diameter, maximum number of roots, and network bushiness between solution pH treatments (Table 19).

Under solution pH 5.5, the root width diameter was higher among pH treatments. However, all other parameters, number of connected components, maximum number of roots, network depth, and network width and network bushiness, showed doubled or tripled improvement at pH 8.0. The regression analysis showed a linear relationship between solution pH and root width diameter, number of connected components, maximum number of roots and a quadratic relationship in network width and network bushiness. The interaction between rootstocks and solution pH was not significant in all the first basic root class (Table 19).

The second root group includes; network area, network perimeter, network surface area, network length, network volume (Table 20). Like the first-group root parameters, there was no significant difference within rootstock in all the second group root parameters. However, a highly significant differences $P \leq 0.001$ were found among solution pH in all parameters. Among solution pH treatments, the highest values of network area, network perimeter, network surface area, and network volume were found at pH 8.0 with exception to higher network length at pH 5.5. The interaction between rootstocks and solution pH showed a significant difference in the average network volume (Table 20). However, when each rootstock was analyzed for regression and correlation individually, each rootstock parameters were correlated differently. (Appendix tables 54-61)

Table 19.Effect of solution pH on root architecture of four Geneva apple rootstocks grown in aeroponics system in 2018 at Geneva, NY.

| Rootstock | Soil pH | Root Width (Diameter mm) | Number of Connected Components | Maximum Number of Roots | Network Depth (cm) | Network Width (cm) | Network Bushiness ratio |
|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | |
| G210 | . | 5.00a [Z] | 7.31a | 36.7a | 175.8a | 128.6a | 1.81 |
| G214 | . | 4.90a | 8.52a | 36.1a | 188.6a | 122.2a | 1.67 |
| G41 | . | 4.98a | 7.07a | 39.1a | 178.7a | 123.3a | 1.70 |
| G890 | . | 4.83a | 8.25a | 34.2a | 188.9a | 108.3a | 1.63 |
| Rootstock significance | | NS | NS | NS | NS | NS | NS |
| - | 5.5 | 5.16a | 3.91b | 31.8b | 175.6b | 85.4b | 1.66a |
| - | 6.5 | 4.95ab | 6.25b | 32.1b | 166.8b | 80.1b | 1.73a |
| - | 8 | 4.68b | 12.88a | 45.1a | 207.1a | 192.3a | 1.7a |
| pH significance | | ** | *** | ** | *** | *** | NS |
| Regression | | L* | L*** | L** | Q* | Q** | NS |
| Interaction means | | | | | | | |
| G210 | 5.5 | 5.30 | 3.46 | 28.8 | 155.3 | 86.2 | 1.79 |
| | 6.5 | 4.95 | 4.88 | 36.1 | 162.8 | 93.0 | 2.00 |
| | 8 | 4.80 | 13.40 | 44.2 | 208.2 | 205.8 | 1.62 |
| G214 | 5.5 | 5.07 | 2.71 | 34.0 | 175.8 | 84.2 | 1.63 |
| | 6.5 | 4.70 | 8.75 | 32.8 | 180.9 | 82.7 | 1.56 |
| | 8 | 4.95 | 13.38 | 41.3 | 207.5 | 194.8 | 1.80 |
| G41 | 5.5 | 5.49 | 4.54 | 31.9 | 179.7 | 96.3 | 1.60 |
| | 6.5 | 5.05 | 3.94 | 30.0 | 151.3 | 69.8 | 1.80 |
| | 8 | 4.52 | 11.94 | 53.2 | 203.7 | 194.2 | 1.67 |
| G890 | 5.5 | 4.90 | 4.72 | 32.0 | 187.0 | 77.9 | 1.63 |
| | 6.5 | 5.10 | 7.39 | 29.6 | 171.7 | 74.7 | 1.57 |
| | 8 | 4.46 | 12.88 | 41.3 | 209.1 | 176.0 | 1.70 |
| Interaction significance | | NS | NS | NS | NS | NS | NS |

[Z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicate treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels, or had a non significat effect, respectively.

Table 20. Effect of solution pH on root architecture of four Geneva apple rootstocks grown in aeroponics system in 2018 at Geneva, NY

| Rootstock | Soil pH | Network Area ($cm^2$) | Network Perimeter (cm) | Network Surface Area ($cm^2$) | Network Length (cm) | Network Volume (L) |
|---|---|---|---|---|---|---|
| Main Effect | | | | | | |
| G210 | . | 34.3a [z] | 151.7a | 141.5a | 96.3a | 2.55a |
| G214 | . | 35.7a | 169.2a | 146.9a | 106.4a | 2.45a |
| G41 | . | 35.5a | 173.4a | 145.2a | 108.1a | 2.38a |
| G890 | . | 33.1a | 158.5a | 136.1a | 99.6a | 2.30a |
| Rootstock significance | | NS | NS | NS | NS | NS |
| - | 5.5 | 30.0b | 127.7b | 122.9b | 155.5a | 2.24b |
| - | 6.5 | 24.8b | 117.3b | 101.4b | 77.6b | 1.71b |
| - | 8 | 48.6a | 241.9a | 201.4a | 73.2b | 3.29a |
| pH significance | | *** | *** | *** | *** | *** |
| Regression | | Q** | Q* | Q** | Q* | Q** |
| Interaction means | | | | | | |
| G210 | 5.5 | 24.5 | 97.6 | 994 | 58.5 | 1.88cde |
| | 6.5 | 25.0 | 117.2 | 1031 | 74.5 | 1.79de |
| | 8 | 53.3 | 237.7 | 2214 | 153.8 | 4.00a |
| G214 | 5.5 | 30.6 | 132.6 | 1259 | 81.7 | 2.21bcde |
| | 6.5 | 28.0 | 138.1 | 1147 | 87.0 | 1.84de |
| | 8 | 48.0 | 232.5 | 1976 | 147.5 | 3.27ab |
| G41 | 5.5 | 34.9 | 133.5 | 1425 | 81.1 | 2.79abcde |
| | 6.5 | 21.8 | 94.0 | 866 | 54.8 | 1.53e |
| | 8 | 48.7 | 278.7 | 2024 | 178.8 | 2.87abcd |
| G890 | 5.5 | 30.2 | 141.6 | 1235 | 85.6 | 2.14bcde |
| | 6.5 | 24.6 | 119.6 | 1010 | 75.8 | 1.68ed |
| | 8 | 45.1 | 217.6 | 1865 | 139.4 | 3.12abc |
| Interaction significance | | NS | NS | NS | NS | * |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a nonsignificat effect, respectively.

The third root parameters group includes; network convex area, network solidity, and network length distribution (Table 21). There was only a significant difference in network solidity within tested four rootstocks, however, no difference was found within the other root parameter in this group. However, highly significant differences at $P \leq 0.005$ were found among solution pH in all parameters. A quadratic relationship was also found in network convex area, and network length distribution and a strong linear relationship in network solidity. However, no significant difference was found in the interaction between solution pH and rootstocks (Table 21).

**3.2. Root nutrients analysis.**

Statistical data from root nutrients analysis showed no significant difference in P, K, Ca, Mg, S, B, Zn, Cu, and Fe within tested rootstock grown in an aeroponics system. Only a significant difference at $P \leq 0.005$ was found in Mn concentration. The mean concentration of P, Ca, and, Mn showed a significant difference within pH treatments (Table 22 &23). Regression analysis showed a quadratic relationship on P, B and Fe and a linear relationship in Ca, Mg, and Fe. The interaction between rootstocks and solution pH showed only a significant difference at $P \leq 0.005$ on root Mn concentration only and no difference was found on other root macro or micronutrients (Table 22 & 23). However, when testing each rootstock individually, different growth paterns were noted (Figure 11, 12).

Table 21.Effect of solution pH on root architecture of four Geneva apple rootstocks grown in aeroponics system in 2018 at Geneva, NY

| Rootstock | Soil pH | Network Convex Area ($cm^2$) | Network Solidity (cm) | Network Length Distribution (cm) |
|---|---|---|---|---|
| Main Effect | | | | |
| G210 | . | 259a [z] | 0.207a | 0.54a |
| G214 | . | 255.5a | 0.213a | 0.60a |
| G41 | . | 249.5a | 0.244a | 0.57a |
| G890 | . | 236.7a | 0.209a | 0.56a |
| Rootstock significance | | NS | * | NS |
| - | 5.5 | 128b | 0.26a | 0.62a |
| - | 6.5 | 116.4b | 0.23a | 0.64a |
| - | 8 | 495.5a | 0.17b | 0.45b |
| pH significance | | *** | *** | ** |
| Regression | | Q** | L*** | Q* |
| Interaction means | | | | |
| G210 | 5.5 | 109.1 | 0.26 | 0.53 |
| | 6.5 | 131.8 | 0.21 | 0.65 |
| | 8 | 533.2 | 0.16 | 0.44 |
| G214 | 5.5 | 121.6 | 0.26 | 0.68 |
| | 6.5 | 136.1 | 0.21 | 0.60 |
| | 8 | 492.1 | 0.17 | 0.54 |
| G41 | 5.5 | 139.1 | 0.28 | 0.57 |
| | 6.5 | 82.8 | 0.27 | 0.70 |
| | 8 | 490.7 | 0.19 | 0.45 |
| G890 | 5.5 | 138.5 | 0.23 | 0.66 |
| | 6.5 | 114.3 | 0.22 | 0.63 |
| | 8 | 470.3 | 0.18 | 0.39 |
| Interaction significance | | NS | NS | NS |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT $p \leq 0.05$. *,**,*** or NS indicates treatment had a significant effect at $P \leq 0.05$ or $P \leq 0.01$ or $P \leq 0.001$ levels or had a nonsignificant effect, respectively.

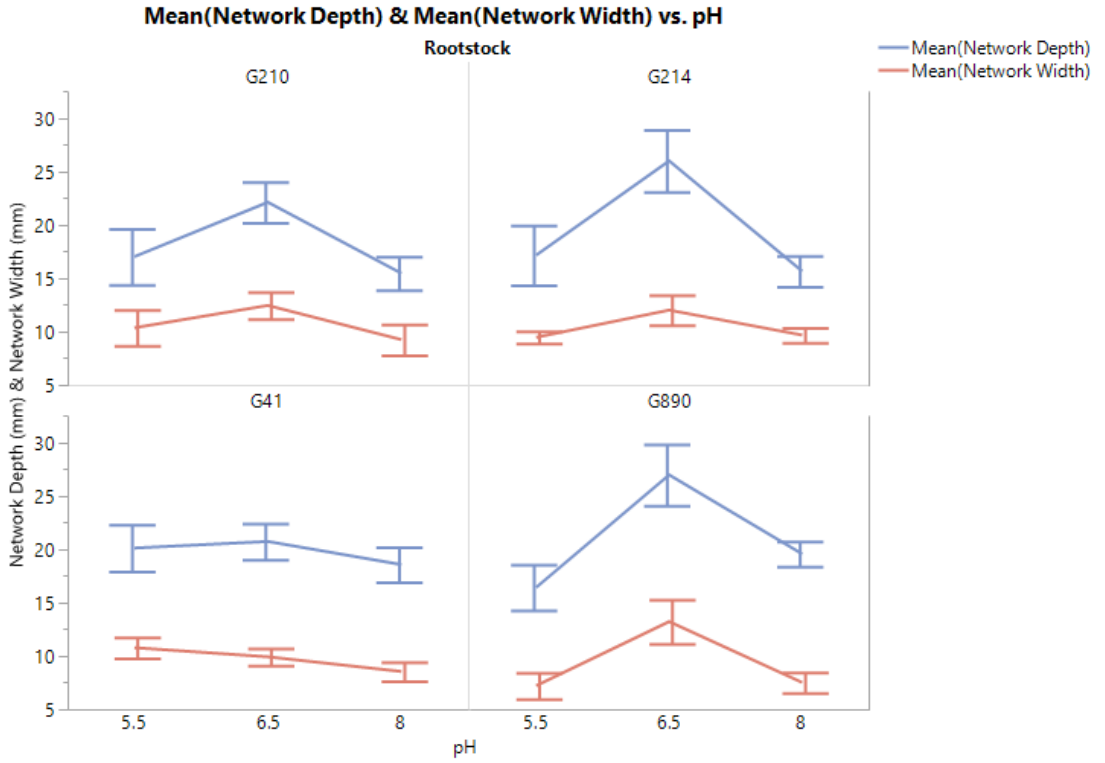


Figure 4. Effect of solution pH on network depth and width in (mm) on four Geneva rootstocks grown in aeroponics system in 2018, bars present stander error.

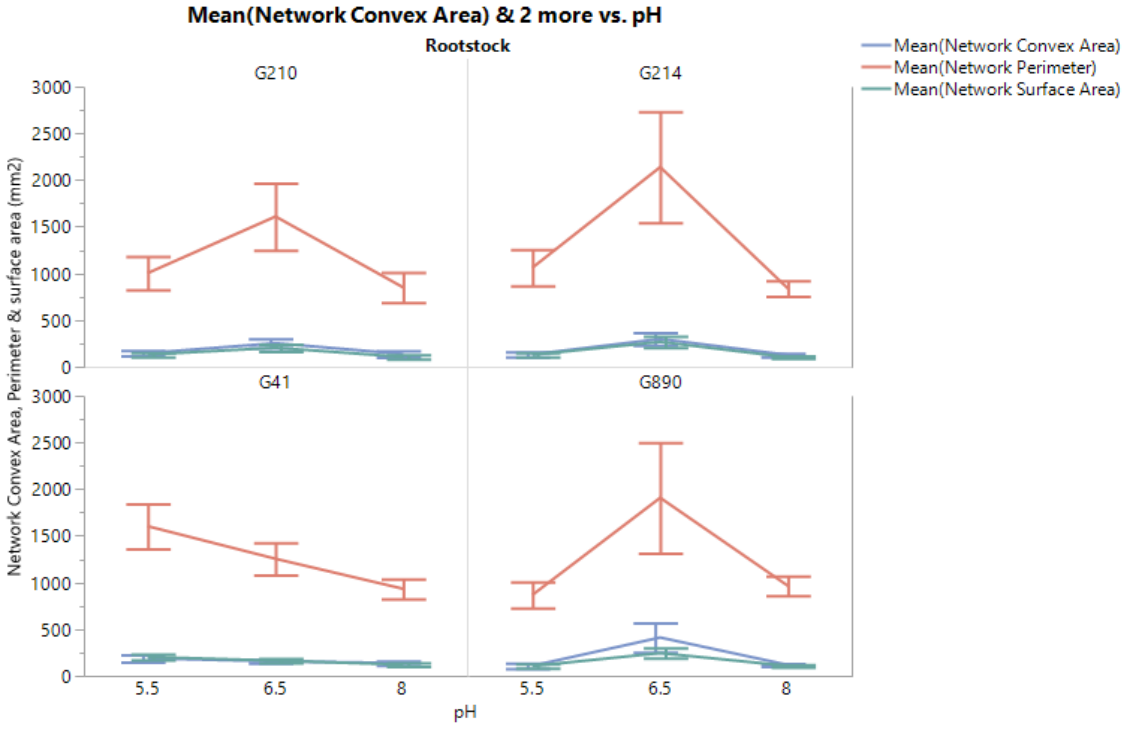


Figure 13 Mean of root network surface area (mm2), length (mm), perimeter (mm) and convex area in (mm2) from 3 cycles aeroponics growth at each ph treatment. Bars represent se of the mean.

Table 22. Effect of solution pH on root nutrients concentration of four Geneva apple rootstocks grown in aeroponics system 2018.

| Rootstock | Soil pH | P (%) | K (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Cu (ppm) | Fe (ppm) | Mn (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | | | | | |
| G210 | . | 3.26 | 1.33b [z] | 0.97 | 0.54a | 1.70a | 48.6a | 35.5a | 11.2a | 60.1a | 59.4a |
| G214 | . | 3.43 | 1.60a | 1.07 | 0.52.8a | 1.77a | 50.0a | 37.3a | 15.4a | 61.2a | 45.5a |
| G41 | . | 3.48 | 1.40ab | 1.08 | 0.61a | 1.65a | 41.5a | 32.1a | 13.9a | 67.9a | 60.1a |
| G890 | . | 3.30 | 1.61a | 1.02 | 0.52a | 1.75a | 55.3a | 39.2a | 14.0a | 49.0a | 45.1a |
| Rootstock significance | | NS | NS | NS | NS | NS | NS | NS | NS | NS | * |
| - | 5.5 | 3.82a | 1.49a | 1.90ab | 0.58a | 1.80a | 51.8ab | 42.4a | 17.5a | 63.6a | 62.7a |
| - | 6.5 | 2.46b | 1.47a | 1.21a | 0.55a | 1.62a | 36.8b | 30.2a | 10.0a | 43.9a | 48.6ab |
| - | 8 | 3.78a | 1.5.3a | 0.80b | 0.49a | 1.75a | 58.4a | 30.2a | 13.4a | 68.8a | 44.3b |
| pH significance | | * | NS | ** | NS | NS | NS | NS | NS | NS | ** |
| Regression | | Q** | NS | L* | L* | NS | Q* | NS | NS | Q* | L* |
| Interaction means | | | | | | | | | | | |
| G210 | 5.5 | 3.74 | 1.17 | 0.84 | 0.53 | 1.68 | 49.5 | 31.9 | 10.2 | 52.1 | 63.3abc |
| | 6.5 | 1.92 | 1.28 | 1.52 | 0.53 | 1.20 | 34.6 | 39.6 | 11.0 | 32.2 | 44.6bcd |
| | 8 | 3.99 | 1.57 | 0.91 | 0.57 | 2.24 | 61.3 | 35.8 | 12.8 | 98.0 | 69.6ab |
| G214 | 5.5 | 3.92 | 1.66 | 1.02 | 0.53 | 1.85 | 54.1 | 53.9 | 22.7 | 85.4 | 54.7abcd |
| | 6.5 | 2.86 | 1.67 | 1.17 | 0.53 | 1.74 | 37.6 | 25.8 | 9.8 | 51.9 | 37.6cd |
| | 8 | 3.50 | 1.47 | 0.74 | 0.44 | 1.72 | 58.4 | 32.1 | 13.6 | 46.4 | 44.1bcd |
| G41 | 5.5 | 4.08 | 1.45 | 1.27 | 0.65 | 1.93 | 48.6 | 32.6 | 22.2 | 64.4 | 81.2a |
| | 6.5 | 2.75 | 1.24 | 1.05 | 0.66 | 1.47 | 27.2 | 24.1 | 7.9 | 54.2 | 70.7ab |
| | 8 | 3.46 | 1.47 | 0.91 | 0.52 | 1.51 | 45.9 | 37.9 | 10.3 | 82.3 | 30.5d |
| G890 | 5.5 | 3.53 | 1.67 | 1.27 | 0.60 | 1.72 | 54.9 | 51.1 | 15.0 | 52.3 | 51.5bcd |
| | 6.5 | 2.29 | 1.57 | 1.14 | 0.51 | 1.89 | 44.0 | 31.6 | 10.8 | 38.3 | 45.8bcd |
| | 8 | 4.13 | 1.60 | 0.68 | 0.46 | 1.63 | 66.8 | 36.8 | 16.3 | 56.8 | 39.1cd |
| Interaction significance | | NS | NS | NS | NS | NS | NS | NS | NS | NS | ** |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicate treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels, or had a non significat effect, respectively.

## 4. Discussion

The aeroponics system, while it is still in the optimization phase, has proven to be an excellent tool to investigate apple rootstock root systems in a controlled environment. In this study apple rootstocks, were grown in aeroponics for apple trees to understand root's dynamics and distribution under varying levels of solution pH. This allowed growing roots in no-rooting substrate other than nutrients-air- mixed in which root are suspended. Repeated monitoring and evaluation of the root system development and the response of solution pH provided sufficient data to run statistical analysis on the root architecture and distribution of apple rootstock roots for the first time in scientific research.

Fine roots are the main components of the root system by which plants absorb water and nutrients. These relatively thin roots, with specific root length to dry weight ratio, form the smallest parts of the root system. Fine roots are an important root's parameter that can be clearly monitored and evaluated in an aeroponics system. This is due to the fact that fine roots are un- suberized and have a high permeability compared to older ones. In apple trees, these roots are generally ≤ 1.0 mm in diameter (De Silva, 1999). However, the diversity of apple rootstocks root systems that can link between certain root properties and their functions is still unclear (Judd et al., 2015).

To better understand the big picture of the root system, multiple parameters were measured in this study by utilizing advances technology in photographing the root's morphology through specialized high definition resolution camera to precisely measure and compare root parameters. This experiment also utilized the more recently developed computer software to increase the accuracy of measuring characteristics of root growth

as well as making it easier and faster. The significance of root size and root morphology for nutrient uptake has been demonstrated in a number of mathematical models and experiments (Boot, 1989).

This experiment monitored the early root formation and development in a period of four weeks. Followed by analyzing images from roots developed in the aeroponics system by evaluated phenotypic difference within solution pH. This short growth cycle was designed to only observe early root formation and fine roots developments. It is encouraging to compare this approach of only evaluating newly formed root with that reported by (Hughes and Gandar, 1993) who found that non-dwarf apple trees showed a semi-elliptic bowl-shaped structure on younger root systems compared to a more layered structure in older root systems. Also, because root's images to be further analyzed based on pixels conversion methods. The fine root dimensions in a high-density root distribution won't be detectable or accurate in converting pixels into millimeters. Result of this study is obviously contrary to long term root growth experiments that might find differences within the same tested rootstocks.

Results from Hughes and Gandar, (1993) suggested that apple roots can penetrate to at least one meter deep in young orchards within 4 growing seasons depending on the orchard's soil properties. However, their data was based on apple grown in a soil substrate.

Rootstocks showed variability in their adaptability for growing in aeroponics in a nutrient-rich misting system. It was noted that some rootstock (G.890) showed better and faster root initiation, while other rootstocks (G.11, G.41) showed 7-9 days delayed root growth under the aeroponics system. Running a multivariate correlation showed

differences within rootstock in response to soil pH and interaction with other root parameters (Tables 19-21).

The statistical results from this study show no difference in root's architecture parameters of the four Geneva rootstocks when processed by Giaroots® software. However, the solution pH was found to affect significantly all root parameters measurements. In this study, four Geneva rootstocks that share the same parents were tested thus, it was not surprising finding similar root behavior. It is possible, however, that the imaging techniques employed were not fine-tuned to detect smaller phenotypic changes between rootstocks confounded in the larger effects caused by the pH effect. This agreed with finding from Fernandez et al who classified root distribution from nine similarly-aged apple rootstock clones into three groups based on total roots and their size (Fernandez et al., 1995). Although, their conclusion was based on using the trench profile method, their finding support similarity of root density within apple rootstocks.

Rootstock G.210 found to have a higher root width diameter, network width, and network bushiness which reflected in their higher root network volume and network convex area. While rootstock G.214 showed a higher number of connected root components which leads to higher network surface area and higher length distribution. This is in agreement with field root morphology observations of the two rootstocks (Fazio, personal comm.)

Root's nutrients analysis was also not affected by different rootstocks uptake capacity and this could be again explained by the similarity of Geneva rootstock root initiation and development rate. Although the morphology of apple rootstocks tested in this study was found better growing at solution pH 8.0, the root nutrient contents were

higher at pH 5.5 (P, Ca, Mg, S, Zn, Cu, and Fe). However, the findings of the current study do not support the previous information about the higher capacity of mineral uptake due to the higher ratio of surface area to volume of available nutrients. This could be explained to the difference in the availability of the nutrients to plant in an aeroponics growing system compared to soil nutrients. It is possible that better growth at pH 8.0 is a direct effect of the root sensing apparatus trying to compensate for low micro-nutrient availability. One of the major influences affecting nutrient uptake by roots in soil is the root length. A high root length favors the uptake capacity and good ability of the plant and vice versa (Boot, 1989).

A possible explanation for this might be due to a short growing time of four weeks where roots absorption and accumulation is higher than translocation or root production.

Since the apple rootstocks were grown in an aeroponic system with non-limiting nutrient supply, the specific root length value may not be as essential for nutrient uptake as in soil-grown with limiting nutrient condition (Zhu and Welander, 1999).

This is the first report on growing apple rootstocks in aeroponics under varying solution pH and thus no previous studies carried similar approaches or published results to compare with. However, most of the studies on apple trees are grown in aeroponics system were conducted to investigate a transformed apple (Zhu and Welander, 1999), testing plant growth regulator translocation (Reed and Buchanan, 1990) or grown to demonstrate hypo-gravity effect (Clawson et al., 2000).

## 5. Conclusion

Root growth and root architecture are frequently left out from horticultural research (Wright and Wright, 2004), investigating root development is challenging research due to the difficulties in implementing a non-destructive root observations tools during plant growing season (Silva and Beeson, 2011).

The present study was designed to determine the effect of varying level of solution pH (pH 5.5, 6.5 and 8.0) on the root's dynamics, distribution, development, and architect of four Geneva apple rootstocks (G.41, G.210, G.214, and G.890) in an optimized aeroponics system for apple trees. The second aim of this study was devised an additional method to investigate the root's architecture, dynamics, and development in controlled soil condition by optimizing minirhizotron systems of four rootstocks (G.41, G 214, G.890 and M.9) under three soil pH treatments (pH 5.0, 6.5 and 8.0).

The aeroponics system

Results from the statistical analysis of the aeroponics study showed no significant difference of root's architecture parameters images within the four Geneva rootstocks processed by GiaRoots® software. Since the tested four Geneva rootstocks shared the same parents thus, it was not surprising finding similar root behavior. However, when each rootstock was investigated individually, each rootstock showed different root's parameters at each solution pH treatments. Also, variation within rootstocks was noted in the timing of root initiation and adaptability to aeroponics growing system.

Solution pH was found to affect significantly all root parameters measurements. Since this was the first attempt to investigate root dynamic and architecture of apple

rootstocks using aeroponics systems, there is more room for improvements to fully optimize the system to accurately understand apple rootstock, root distribution, and dynamics. Implementing a non-destructive rot monitoring system such as aeroponics, allow better comparison and provide molecular evidence when compared with QTL and mineral nutrient traits (Fazio et al., 2018).

Evaluating other apple rootstocks based on their size class would probably show differences within tested rootstocks. In this study, we could not compare it with other results since it was a unique study and no similar root architecture study was found in the literature that implemented an aeroponic system to investigate apple rootstock architecture, dynamic, and distribution.

Evaluating rootstocks developed in micropropagation would present a better explanation of apple rootstocks adaptability for growing apple trees in aeroponics system and will assess improved understanding of the root's architecture based on nutrient uptake from the aeroponic system.

As the current study was conducted in the aeroponic experimental system and the plants were still at a very young age considering the apple as a perennial plant, it is necessary to verify experiment's results under similar conditions both in the greenhouse and in the field to further evaluate these root morphological characteristics of apple rootstocks before a final conclusion can be drawn.

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# CHAPTER FOUR

## Evaluating the Dynamics of Root development and distribution of Four Apple Rootstocks in Response to Varying Soil pH Grown in Minirhizotron system.


### *Abstract*

Most of apple rootstock assessments have been conducted to the above-ground parts of the tree, while below-ground parts are still a mystery. Those evaluations are also long, expensive, destructive and laborious. Extensive studies on other plant species show that root architecture influence rhizosphere nutrient uptake. Understanding the root's distribution and architecture of apple rootstock would provide a precise recommendation for rootstock selection.

However, implementing new techniques aided with technologies would speed up the assessment and support the understanding of how rootstocks will perform in orchard conditions?

This study devised an additional method to investigate the root's architecture, dynamics, and development in controlled soil condition by optimizing minirhizotron systems built in Geneva, NY. Four rootstocks (G 214, G.41, G.890, and M.9) were used in three soil pH treatments (Low: 5.0, Medium: 6.5 and High: 8.0). Twelve rhizoboxes with dimension 1.2m x 1m x 0.8m were equipped with transparent tubes arranged in hexagon arrangement to facilitate the use of narrow gauge $360^0$ Root Imager®. This growing system allows repeatedly sampling and evaluating root's development in a non-destructive way. Root images were taken weekly to measure root's length and

elongation. The high-resolution images were then processed by RootSnap® software for further analysis and rootstock comparison.

The statistical data shows no significant difference in all root parameters within the tested four apple rootstocks. Rootstock G.214 was found to produce a higher number of root and longer root length. Whereas rootstock G.890 produced better root volume and higher root area.

Within soil pH treatments, significant differences were found in the root count, total root length, and the total root area. At soil low pH, the root count, root length, root volume, and root area were higher than the other tested soil pH treatments. While the average root diameter, average root length, average root area, and the average root volume were higher at high pH. As predicted a strong correlation was found between root count and the total root length and between the root volume and the root area and diameter.

## 1. Introduction.

Minirhizotrons represent another approach to study root interaction and development in soil growing medium. Rhizoboxes were designed and installed specifically for apple trees by considering their potential root expansion and distribution. The growing medium pH was pre-adjusted to a range of soil pH to measure the response of apple rootstocks at each pH levels. These rhizoboxes are equipped with multilevel observation tubes and arranged to accommodate apple root's normal growth. In conjunction with a 360◦ root scanner, high-resolution images will enable root analysis software to quantify changes in root length, expansion rate, distribution, type, and turnover. Such vital data could provide a more precise recommendation of favorable soil condition and optimal pH for each tested rootstock.

These tools would provide a real-time monitor to growth changes in terms of root architecture and response to the rhizosphere environment.

### 1.1. Significant of the study & justification

The root of apple rootstock, the little unknown fact about the apple tree, yet fewer researches were dedicated to an important part that contributes a lot in understanding the characterization of apple tree's developments and growth. Utilizing a nondestructive method will help to remove lots of ambiguity and clear up the critical information about the overall health and behaviors of apple rootstock in different growth conditions just equal to understand the aboveground parts.

Another nondestructive method that has been used for studying the root's architect, dynamics and developments are proceeded by optimizing minirhizotron

system. Four rootstocks (G 214, G.41, G.890 and M.9) were used in three soil pH treatments (pH 5.0, 6.5 and 8.0). The high-resolution images produced by root imager were then processed by RootSnap® software for analysis and rootstock comparison.

### 1.2. Objectives:

This study was set to obtain the following: 1. Optimizing a root monitoring system to serve as a scientific tool to study apple rootstocks in varying soil's pH. 2. Monitor and correlate fine root production and turnover rate in response to differences in soil pH. 3. Compare rootstocks root distribution and elongation rate in varying soil pH by calculating the growth change from the processing of root images. 4. Evaluate root behavior response after grafting with Honeycrisp scion cultivar. 5. Assess the nutrient uptake efficacy under pH treatments.

Measurable observation of a plant's root system in a non-destructive way can provide more accurate data with regards to root growth and development. Experimentation of new methods that facilitate in-vivo monitoring in the reproducible controlled growing system was an essential component of this study. This allowed repetitive sampling and observation without disturbing the growth flow and normal distribution.

## 2. Materials & Methods

### 2.1. Minirhizotron design and setup.

**Minirhizotron observation bins (Rhizobox)**

High-density polyethylene structural foam vented sidewalls fruit bins model MACX48 (Decade Products MI, USA) were used in this experiment as Rhizobox. Twelve rhizoboxes with dimensions 122cm x 102cm x 79cm (Width x length x depth) were side drilled using a 8cm hole saw (Figure 13).

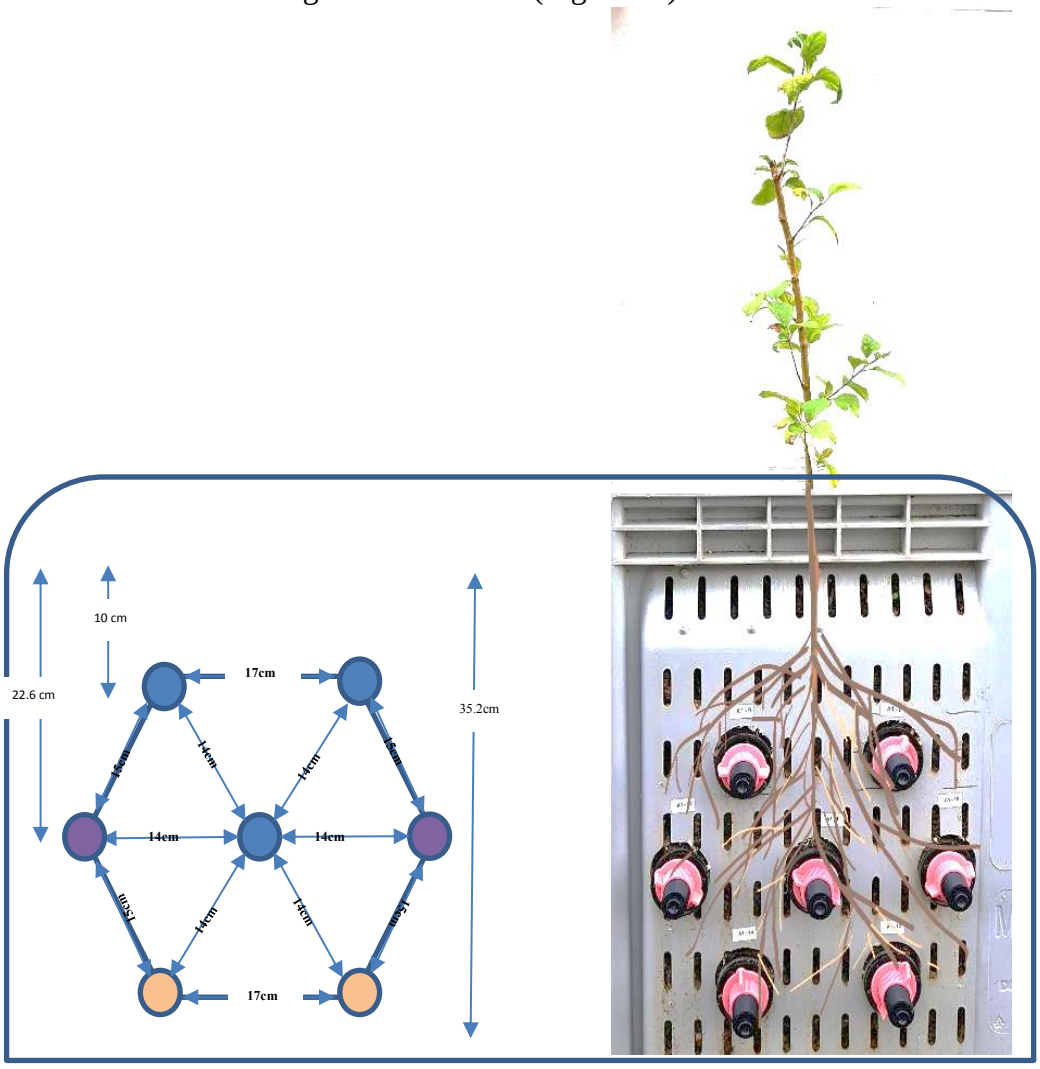


Figure 14. Distribution of the minirhizotron observation tubes unit.

Each rihzobox was equipped with 14 color-labeled transparent polycarbonate observation tubes. Tubes were 5cm inner diameter and arranged in two hexagon arrangements. OTs were designed to be in the center of each rihzobox to maximize interaction with root growth. The OTs were in three levels; where each level maintained a fixed distance from the inner bottom of the rihzobox. The first lower level (tube 3,4) was at 13cm and the second middle level (tubes # 2,5,7) was at 23cm and the third upper level was at 33cm. The distance from outer tubes (tubes #1,2,3,4,5,6,) to the center tube (tube # 7) was fixed to 14 cm. While the distance between the two lower and upper tubes (tubes # 1,6 & 3,4) was maintained as 17 cm and the distance between all other OT was 15cm. Each OT was 125cm long and capped with rubber seal plunger permanently from one side and removable plunger from the other side to allow insertion of the root imager. Observation tubes were divided into 6 observation windows and marked with color labeled. Each observation is equal to the root imager field of view.

### 2.2. Soil testing

Top screened soil from local soil supplier (Cayuga Compost P&S Excavating LLC, Trumansburg, NY) which was previously tested for pH buffering capacity and nutrient availability was used in this experiment. The soil sample was then further screened in a 1.27cm mesh screen and sent for nutrient analysis. The soil was then tested for pH buffering capacity by adjusting the pH to acidic and alkaline by addition of elemental sulfur or calcium carbonate respectively. Samples were then monitored for one year in lab and soil pH was evaluated and observed. The desired soil's pH range was achieved by optimizing the correct mix combination of soil: perlite: sulfur/calcium carbonate.

### 2.3. Soil preparation

Original soil's pH was 7.1 and was adjusted to 5.0, 6.5 and 8.0. +/- 0.3. Perlite was added to the soil mixes on ratio 1:4 to improve soil structure and aeration when the mixture is used in pots. A front loader bucket was used to prepare and mix the soil mixes to maintain a homogenous mix. Mixed with perlite in 1:4 ratio and added elemental sulfur (99.9% sulfur Duda Energy LLC, Decatur, AL) to lower pH (Acidic) or calcium carbonate (CalCarb AC3, Mississippi lime) to raise the pH (alkaline). The adjusted soil medium was used to fill the rhizobox. The adjusted soil was fully compacted with repeated flood irrigation before planting. Soil samples were collected from each adjusted pH soil and prepared for a nutrients analysis.

Samples were digested with nitric and perchloric acids using the Vulcan 84 automated digestion system. (Questron Technologies Cor. Mississauga Ontario Canada). About .30 to 1.0 grams of sample were weighed into 50ml Teflon containers plus .25 ml of 80 ug per ml of yttrium. This is used as an internal standard. The digestion system automatically using syringe pumps added 5.0 ml of 67-70% Omni Trace nitric acid plus 3.0 ml of environmental grade 70% perchloric acid from GFS chemicals Columbus Ohio. The samples are heated to 110℃ over 40 minutes and held for 60 minutes. The temperature is increased to 160℃ over 20 minutes and held for 15 minutes. An additional 1.0 ml of nitric acid is added and the samples heated an additional 20 minutes at 160℃. After cooling ,20.0 ml of 18meg water is added. The solutions are then analyzed using an axial viewed ICP-OES. (Spectro Arcos FHE12 made in Kleve Germany).

All the results were verified for accuracy by inspecting the spectral display for each element reported for all the samples. Table 1 lists the approximate method detection limits. This was determined by multiplying the instrument detection limits by the dilution factor which is about 67 for a 0.3g sample. Because there are no certified reference materials that are typical of these worm samples so several Results from NIST certified samples were included to validate the methods of analysis used. All nutrients analyses were conducted at Cornell Nutrients Analysis Laboratory, NY, USA

Table 23.Nutrients analysis of soil used in Rhizobox after one growing season (fall 2017)

| **Element** | **Value at pH 5.0** | **Value at pH 6.5** | **Value at pH 8.0** |
|---|---|---|---|
| Organic matter (%) | 4.23 | 4.20 | 4.48 |
| pH | 5.19 | 6.37 | 8.46 |
| Moisture (%) | 1.36 | 1.23 | 0.86 |
| Aluminum (mg/Kg) | 7.08 | 6.30 | 5.83 |
| Boron (mg/Kg) | 1.64 | 1.58 | 1.73 |
| Copper (mg/Kg) | 0.10 | 0.12 | 0.12 |
| Iron (mg/Kg) | 9.55 | 13.06 | 13.75 |
| Calcium (mg/Kg) | 15042 | 17451 | 21330 |
| Magnesium (mg/Kg) | 724.54 | 489.21 | 432.58 |
| Manganese (mg/Kg) | 37.60 | 53.02 | 71.30 |
| Potassium (mg/Kg) | 588.33 | 614.00 | 661.21 |
| Phosphorus (mg/Kg) | 61.02 | 65.30 | 61.35 |
| Sodium (mg/Kg) | 196.69 | 185.79 | 190.57 |
| Sulfur (mg/Kg) | 7874 | 4998 | 66.17 |
| Zinc (mg/Kg) | 1.35 | 1.43 | 1.51 |

### 2.4. Plant materials.

The plant materials used in this experiment were a 1-year old, non- grafted apple rootstocks supplied by commercial nursery (North America Plants. Inc USA). Four commercially apple rootstocks were selected to be investigated by using rootstocks; G.890, G.41 and G.214 and M.9. The rootstocks' genetic background, country of origin, and specific characteristics are listed in Table 29.

Prior to planting into the minirhizotron bins, plants were grown on potting mix soil for one year with regular nursery maintenance.

Table 24. Apple rootstocks and their parents and characteristics

| Rootstocks | Parents | Breeding program | Specific Characteristics |
|---|---|---|---|
| G.890 | Ottawa 3 x Robusta 5 | Cornell Geneva | Semi-dwarfing |
| G.214 | Ottawa 3 x Robusta 5 | Cornell Geneva | Dwarf |
| G.41 | M.27 x Robusta 5 | Cornell Geneva | Dwarf |
| M.9-T337 | *Malus pumila Mill.* var. *paradisiaca* (L.) C.K. Schneider | East Malling Research | Dwarf |

**2.5. Experiment layout and treatment.**

Twelve bins were used in this setup with four bins per treatment. Four apple rootstocks were tested (G.214, G.41, G.890, and M.9) with four replicates and two trees per replicate. The CI-602 root imager was used to take a weekly image of root's developments from the spring till fall. However biweekly images were taken during the winter. The experiment design was following the complete randomized block design.

**2.6. Transplanting and maintenance.**

A preliminary assay was conducted to screen and evaluate individual plants based on consistent height and stem diameter. When the stem's diameter was 10-12mm, plants were removed from the potting soil and roots were carefully cleared from the previous potting soil. On July 13$^{th,}$ 2017, selected trees were planted vertically while maintaining the root crown to be at least 10 cm above the center tube (tube#7). Rhizoboxes were placed in a fenced pot lot area in Cornell AgriTech, Geneva, NY. Regular irrigation and pest/disease management were carried out throughout the experiment. However, no fertilization or any soil amendments were applied so no other interaction can skew root developments. During the winter, the rhizoboxes were moved to the storage cellar maintained at 4C° to prevent cold damage till the next spring.

### 2.7. Spring budding by Honeycrisp scion.

During the spring of 2018, all rootstocks were budded with Honeycrisp scion variety. Each rootstock was budded by two buds 15cm above the soil level. Buds were allowed to grow, and rootstocks were topped. During this budding and scion growth, the root growth and behavior were noted and observe to investigate any changes from previous non-grafted rootstock root's growth. As soon as the budded scion started to grow, the rootstock was topped to allow the scion Honeycrisp only to grow.

### 2.8. Roots scanning.

The $360^0$ root scanner from Cid Biosciences (CI-602 Narrow Gauge Root Imager ®, CID Bio-Science, Inc) was used in this experiment. The OT endcaps were opened, and tubes were cleaned and dried from moisture using a steel rood with a microfiber cloth. This was done before every scan session to ensure a clear image and to protect the root imager from damage by water and soil debris. Root scanner was inserted manually to each observation window inside the OT, while the scanner rotates/ revolve automatically to take a $360^0$ image from each observation window. The scanning resolution was 600 dpi and it took 30mintues to scan each rhizobox.

Since root growth was not observed and not predicted in the lower OT levels in the initial scan sessions, a scanning scheme was divided into 3 intervals per season. The scanning procedure was first by scanning level 1 for the first 4 months of the growth. Followed by scanning level 1 and 2 for the next four months and then scanning all level after that. Each scanning session was labeled with the date, rhizobox Id, pH treatment, OT Id and observation window number. This was done to facilitate organizing a large

number of images and to systemically process images in RootSnap® software.

Root scanning was done weekly from 11 Aug. 2017 to 17 Nov. 2017 and once every 2 weeks during dormancy (24 Nov. to 17 May 2018) and then weekly from spring till fall (25 May to 23 Nov 2018). There was a total of 42 observations during the experiment period and skipping 3 months of observation during scanners service.

### 2.9. Data collection and root image processing

All high-resolution images were analyzed, and the root data were measured using the RootSnap® software image analysis software (CID Bioscience, Inc). Each image from each observation window of each OT was analyzed manually using the software by tracing every root and fine root. Although auto-tracing was incorporated in the software, it was found to over-estimate root extension, thus manual tracing was practiced. The root length, surface area, diameter, volume, and a number of tips were measured using RootSnap® and was exported in excel spreadsheet for statistical analysis.

### 2.10. Leaf samples

During July, five mid-position leaves were collected from all trees individually for nutrients analysis. Samples were washed thrice with DI water and then oven dries at 70℃ for 10 days. Samples were then ground and placed in paper bags. All nutrients analyses were conducted at Cornell  Nutrient Analysis Laboratory using the same protocols mentioned earlier in base nutrients analysis in the previous section.

## 3. Results

### 3.1. Rhizobox and images processing

Root images were taken weekly during the active growing season from spring to the fall and monthly during the dormant growth during winter till early spring. There were 16 observation sessions during 2017 and 34sessions during 2018. Each observation session required 6 hours during the early growth stages and up to 10 hours when scanning all observation tubes. Total root images taken in 2017 were 16,128 images and in 2018 there were 36,288 images with a total of 52,416 images. All images were identified with rhizobox number, soil pH treatment, observation tube number, and observation window number. This facilitated identifying corresponding rootstock images during images processing. Root tracing was only considering new root developed after the last session to avoid double calculation. The experiment was conducted for 511 days during 2017-2018 in Cornell AgriTech, Geneva, NY.

All images were processed using RootSnap® software image analysis software (CID Bioscience, Inc. The software allows automatic detection of root and automatic tracing of root extension by segmenting background and foreground image pixel. However, the soil mix used in the rhizobox were mixed with perlite and that would interfere with automatic reading. Also, after several months, the observation tube materils started to interact with the soil particles and some observation windows were not as clear for automatic root tracing. For those reasons, manual root tracing was conducted using high-resolution computer screen while paying attention to small fine root.

Each observation tubes was divided into seven observation windows and using

3 observation windows for each rootstock. The middle window was left as blank to avoid root crossing. Thus, in statistical analysis, data from each observation tubes were grouped and the total sum was calculated. Data were analyzed using SAS 9.4 statistical software and JMP 14.0 PRO (SAS Institute Inc. Cary, NC, USA).

The image processing software was selected to export the following root parameters data; root count, total root length, total root volume, total root area, average root diameter, average root length, average root area, and average root volume.

### 3.2. Root growth

The statistical data shows no significant difference in all root parameters within the tested four apple rootstocks. Rootstock G.214 was found to produce a higher number of root and longer root length. Whereas rootstock G.890 produced better root volume and higher root area (Table 25).

Within soil pH treatments, significant differences were found in the root count, total root length, and the total root area. At soil low pH, the root count, root length, root volume, and root area were higher than the other tested soil pH treatments. While the average root diameter, average root length, average root area, and the average root volume were higher at high pH. Quadratic regression was found only under root count and toot root length while no correlation was found on the other root parameters (Table 25).

There were three levels of observation depth in each rhixobox; 10cm, 22.6cm and 35.2. Statistical analysis showed a highly significant difference at $P \leq 0.001$within depth level and all root parameters. Days after planting shows also a highly significant difference at $P \leq 0.001$ within rootstock and root parameters (Table 25).

When testing the effect of the interaction between the apple rootstocks and the soil pH, the highly significant difference at P≤ 0.001was found on root count and total root length.

The root's images analysis shows no correlation between soil pH and root's morphological parameters. However, a negative correlation was found between the average root diameter and day after planting. As predicted a strong correlation was found between root count and the total root length and between the root volume and the root area and diameter (Table.28). However such correlation was noted in between root parameters within rootstocks. (Appendix table 63).

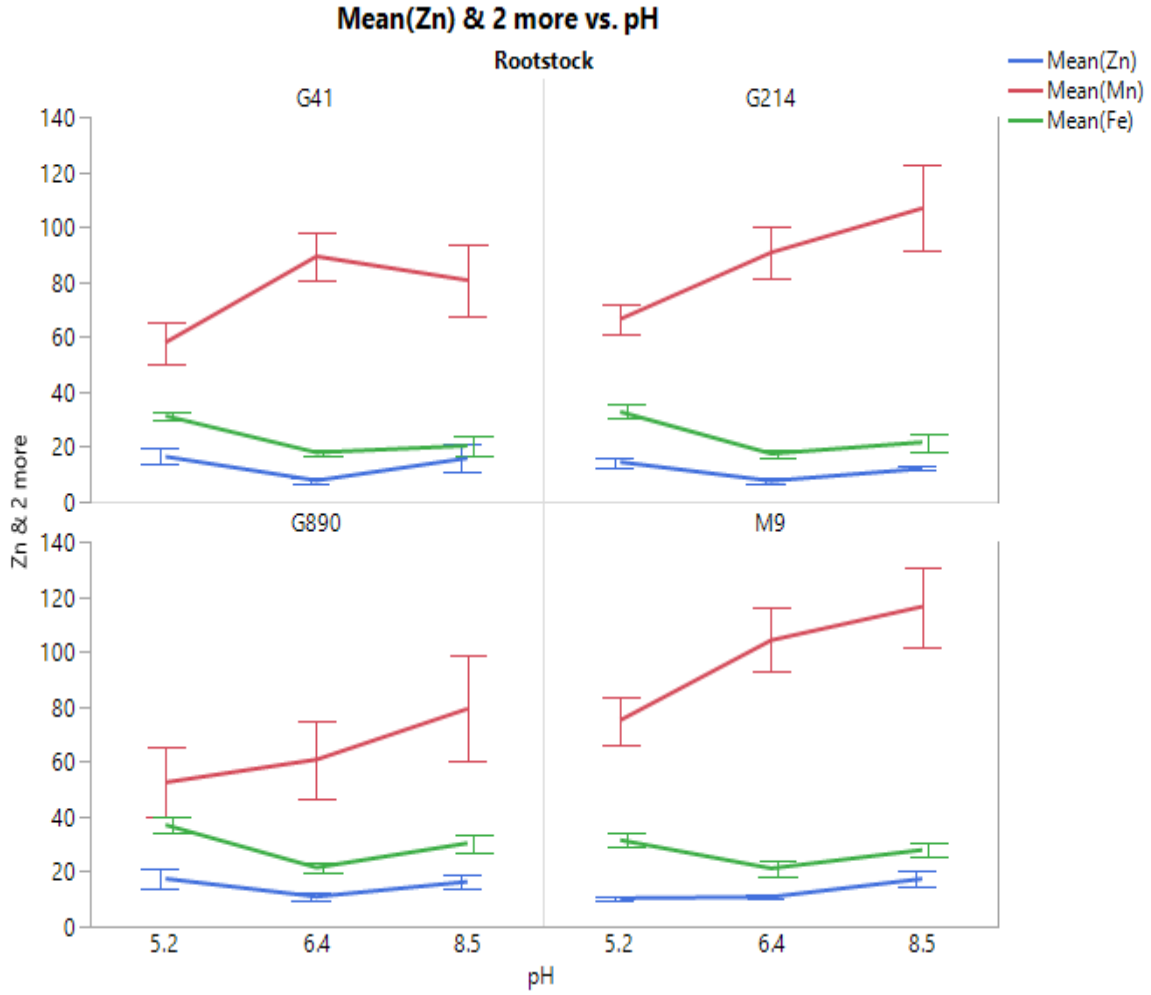


Figure 15. Effect of soil pH on Honeycrisp leaf zinc, manganese and iron concentration of four apple rootstocks displayed by rootstocks grown in minirhizotron 2018.

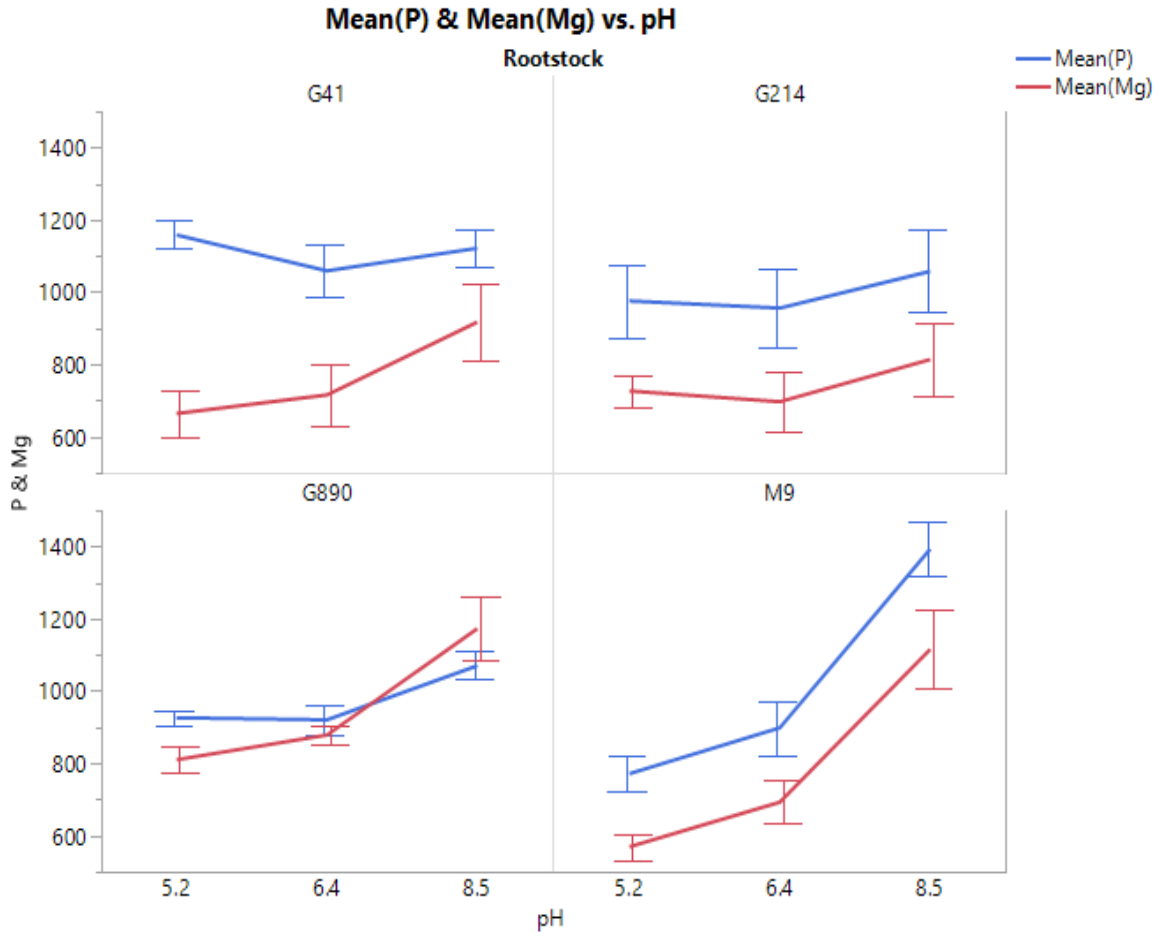


Figure 16. Effect of soil pH on Honeycrisp leaf phosphorus, and magnesium concentration of four apple rootstocks displayed by rootstocks grown in minirhizotron 2018

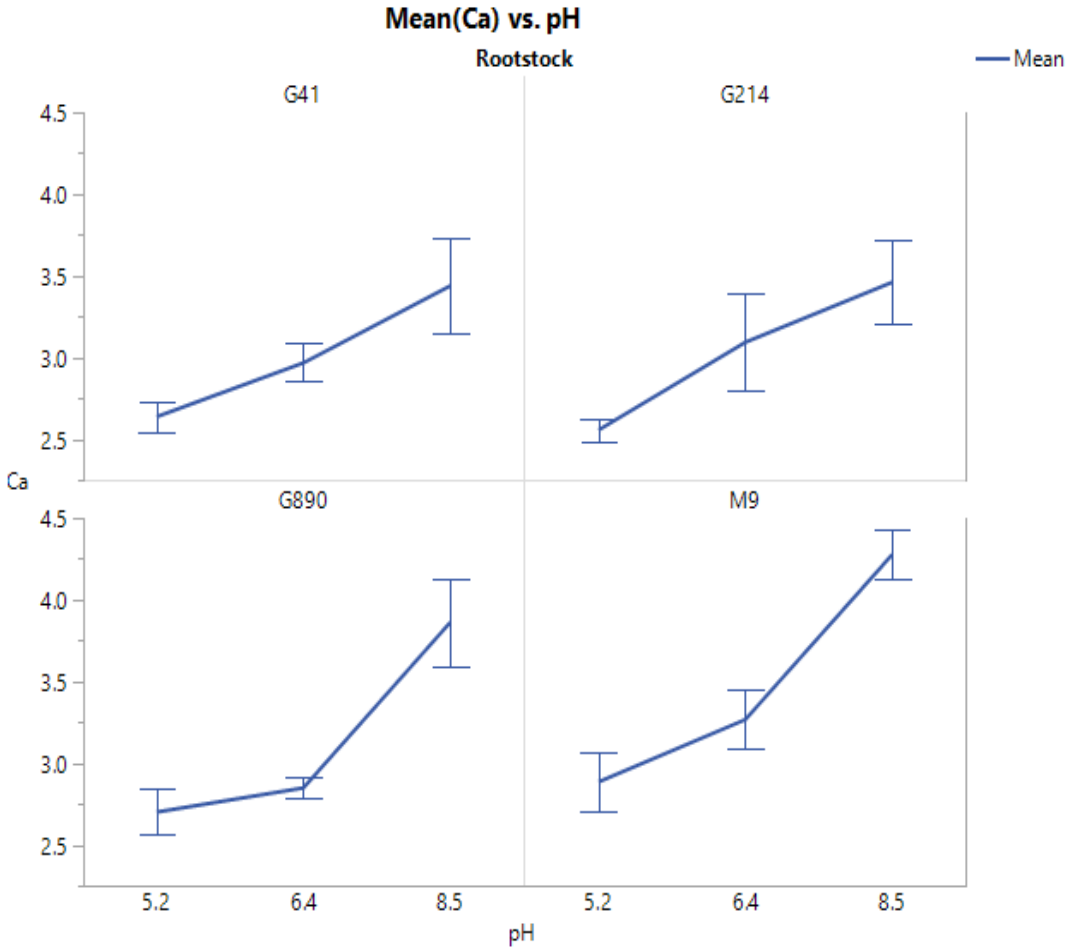


Figure 17. Effect of soil pH on Honeycrisp leaf Calcium concentration of four apple rootstocks displayed by rootstocks grown in minirhizotron 2018.

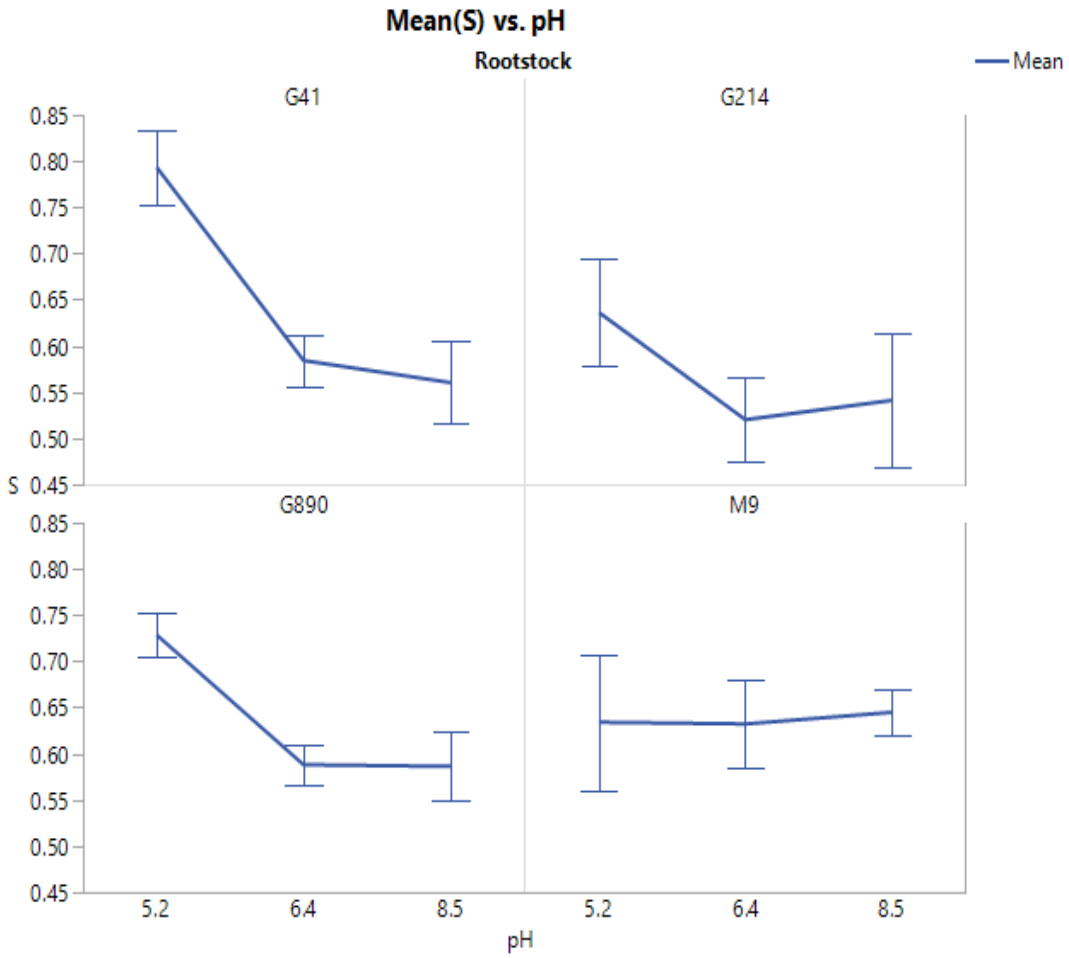


Figure 18. Effect of soil pH on Honeycrisp leaf sulfur concentration of four apple rootstocks displayed by rootstocks grown in minirhizotron 2018.

### 3.3. Leaf nutrients analysis

Leaf samples from the grafted Honeycrisp scion were harvested in 2018. The statistical nutrients analysis showed a significant difference in leaf contents of calcium, magnesium, copper, manganese, sodium, iron and carbon within rootstocks. A highly significant difference was found in mean percentage at $P \leq 0.001$. Also, a highly significant difference within rootstocks was shown in the Na and Carbon concentration at $P \leq 0.001$. However, N and P, S, B and Zn showed no significant difference within rootstocks in (Table.24).

Rootstock M.9 was found to acquire higher leaf contents of phosphorus, calcium, sulfur, manganese, and sodium in the minirhizotron setup while rootstock G.41 was found to have better values of nitrogen, boron, and copper.

When comparing leaf nutrient contents within soil pH treatments, a highly significant difference at $P \leq 0.001$ was found in phosphorus, calcium, magnesium, boron, sodium, iron and carbon. Additionally, a significate difference was also found in sulfur, zinc, and manganese.

The leaf content of nitrogen, phosphorus, calcium, magnesium, boron, zinc, manganese was highest at high pH. However, the content of sulfur, copper sodium and iron was higher at soil low pH treatments (Table.26).

The interaction between soil pH and apple rootstock in leaf contents was found a significant difference in nitrogen, phosphorus, copper, sodium, and carbon. However, no significant difference was found in the interaction within the other leaf nutrients.

The regression correlation showed a linear positive relationship between soil pH treatment and phosphorus, calcium, boron, and manganese. A quadratic relationship was found in the leaf content of magnesium, sulfur, zinc, sodium, iron, and carbon.

Nutrient correlation analysis shows a positive correlation between soil pH and leaf calcium and magnesium. Magnesium was found to correlate positively with nitrogen, phosphorus, and potassium (Table 26). Leaf iron was also found to positively correlate with sulfur and zinc.

When plotting leaf nutrients at each rootstock individually, similar patterns were noted within rootstock. However, nutrients contents were different within soil pH treatments. Leaf manganese and zinc show similar percentage pattern on all rootstocks where higher percentages were observed at low pH and high pH and slightly lower at medium pH treatments (Figure.21). The means leaf percentage of phosphorus and magnesium were also following similar patterns in all rootstocks. However, phosphorus and magnesium leaf content was significantly higher at high pH in rootstock M.9 (Figure.22).

Leaf potassium was significantly higher at medium pH and high pH treatments while sulfur was higher at low pH and lower at medium pH and high pH treatments in all rootstocks (Figure 23,24).

Table 25. Effect of Soil pH on root growth of four Geneva apple rootstocks grown in minirhizotron system in 2018 at Geneva, NY

| Rootstock | Soil pH | Root count | Total root length | Total root volume | Total root area | Average root diameter | Average root length | Average root area | Average root volume |
|---|---|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | | | |
| G214 | . | 13.8a[z] | 316.3a | 899.6a | 1165a | 1.86a | 31.0a | 180a | 194a |
| G41 | . | 10.1a | 255.3a | 1182.9a | 1141a | 2.13a | 30.2 | 203a | 260a |
| G890 | . | 11.1a | 294.4a | 1354a | 1342a | 1.61a | 29a | 162a | 173a |
| M9 | . | 9.74a | 252.8a | 1101a | 1209a | 1.78a | 29.1a | 183a | 207a |
| Rootstock significance | | NS | NS | NS | NS | NS | NS | NS | NS |
| - | 5.2 | 13.5a | 331a | 1221a | 1419a | 1.73a | 28.1a | 171a | 185a |
| - | 6.4 | 12.1a | 304a | 1100a | 1199a | 1.91a | 31.3a | 182a | 207a |
| - | 8.5 | 5.4b | 149b | 1056a | 896b | 1.91a | 30a | 203a | 252a |
| pH significance | | NS | NS | NS | * | NS | NS | NS | NS |
| Regression | | Q* | Q** | NS | NS | NS | NS | NS | NS |
| Depth | | ** | ** | *** | *** | *** | ** | *** | *** |
| Day after planting | | *** | *** | *** | *** | *** | *** | *** | *** |
| Interaction means | | | | | | | | | |
| G214 | 5.2 | 21.9 | 490 | 1046 | 1694 | 1.64 | 27.1 | 157 | 169 |
| | 6.4 | 11.8 | 274 | 1085 | 1080 | 2.29 | 35.0 | 212 | 256 |
| | 8.5 | 5.4 | 133 | 359 | 535 | 1.42 | 29.9 | 158 | 123 |
| G41 | 5.2 | 10.0 | 259 | 1263 | 1236 | 2.10 | 30.6 | 212 | 255 |
| | 6.4 | 12.7 | 308 | 925 | 1135 | 2.01 | 28.4 | 152 | 159 |
| | 8.5 | 6.2 | 168 | 1410 | 962 | 2.35 | 32.2 | 262 | 420 |
| G890 | 5.2 | 11.9 | 307 | 1635 | 1566 | 1.69 | 27.5 | 174 | 205 |
| | 6.4 | 12.8 | 350 | 1370 | 1410 | 1.68 | 30.7 | 170 | 177 |
| | 8.5 | 4.7 | 127 | 710 | 691 | 1.28 | 27.5 | 119 | 97 |
| M9 | 5.2 | 11.8 | 298 | 911 | 1248 | 1.41 | 27.0 | 130 | 95 |
| | 6.4 | 11.1 | 278 | 960 | 1134 | 1.68 | 30.6 | 192 | 235 |
| | 8.5 | 5.2 | 159 | 1543 | 1258 | 2.35 | 30.0 | 245 | 320 |
| Interaction significance | | *** | *** | NS | NS | NS | NS | NS | NS |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels, or had a non-significant effect, respectively

Table 25.Effect of soil pH on Honeycrisp leaf nutrients concentration of eight apple rootstocks grown in minirhizotron 2018 in Geneva.

| Rootstock | Soil pH | N (%) | P (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Cu (ppm) | Mn (ppm) | Na (ppm) | Fe (ppm) | C (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Main Effect | | | | | | | | | | | | | |
| G214 | . | 0.78a [z] | 1.00ab | 3.04b | 0.75b | 0.57a | 13.9a | 11.4a | 6.60b | 87.9a | 34.2bc | 23.9b | 46.9a |
| G41 | . | 0.86a | 1.11a | 3.03b | 0.77b | 0.64a | 14.0a | 13.0a | 8.19a | 77.3a | 24.0c | 22.6b | 46.7b |
| G890 | . | 0.78a | 0.97b | 3.14b | 0.95a | 0.63a | 12.4a | 14.9a | 6.49b | 64.1b | 40.2b | 29.6a | 46.6b |
| M9 | . | 0.77a | 1.02ab | 3.48a | 0.79b | 0.64a | 13.1a | 13.00a | 7.30ab | 98.5a | 89.1a | 26.8ab | 46.5b |
| Rootstock significance | | NS | NS | * | ** | NS | NS | NS | * | ** | *** | * | *** |
| - | 5.0 | 0.80a | 0.95b | 2.70a | 0.70b | 0.70a | 11.5c | 14.6a | 7.36a | 63.13b | 70.9a | 33.1a | 46.5b |
| - | 6.5 | 0.78a | 0.96b | 3.04b | 0.75b | 058b | 13.3b | 9.19b | 7.00a | 86.3a | 32.1b | 19.5c | 46.8a |
| - | 8 | 0.81a | 1.16a | 3.76a | 1.00a | 0.58b | 15.2a | 15.4a | 7.09a | 95.7a | 38.9b | 25.1b | 46.7a |
| pH significance | | NS | *** | *** | *** | ** | *** | ** | NS | ** | *** | *** | *** |
| Regression | | NS | L** | L*** | Q*** | Q** | L** | Q** | NS | L** | Q** | Q*** | Q** |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non-significant effect, respectively.

Table 26. Effect of the interaction between rootstocks and the soil pH on Honeycrisp leaf nutrients concentration of eight apple rootstocks grown in minirhizotron 2018 in Geneva.

| Rootstock | Soil pH | N (%) | P (%) | Ca (%) | Mg (%) | S (%) | B (ppm) | Zn (ppm) | Cu (ppm) | Mn (ppm) | Fe (ppm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Interaction means | | | | | | | | | | | |
| G214 | 5.0 | 0.82 | 0.98bcde | 2.56e | 0.73cde | 0.64bc | 11.70cd | 14.44abc | 8.21abcd | 66.5 | 32.7ab |
| | 6.5 | 0.76bcd [z] | 0.96bcde | 3.09cde | 0.70de | 0.52c | 15.46ab | 7.60c | 6.16de | 90.7 | 17.5d |
| | 8 | 0.76bcd | 1.06bcd | 3.46bc | 0.81cd | 0.54c | 14.55abc | 12.04abc | 5.44e | 106.8 | 21.6cd |
| G41 | 5.0 | 0.95a | 1.16b | 2.64e | 0.66de | 0.79a | 13.53abcd | 16.41ab | 9.76a | 58.0 | 31.2ab |
| | 6.5 | 0.86abc | 1.06bcd | 2.97cde | 0.72cde | 0.58c | 12.17bcd | 7.71c | 8.51abc | 89.3 | 17.9d |
| | 8 | 0.78abcd | 1.12bc | 3.44bc | 0.92bc | 0.56c | 16.62a | 15.86ab | 6.45cde | 80.6 | 20.4d |
| G890 | 5.0 | 0.81abcd | 0.93cde | 2.70e | 0.81cd | 0.73ab | 11.25cd | 17.38a | 6.10de | 52.4 | 37.0a |
| | 6.5 | 0.75cd | 0.92cde | 2.85de | 0.88cd | 0.59c | 12.23bcd | 10.88abc | 5.90e | 60.7 | 21.5cd |
| | 8 | 0.77abcd | 1.07bcd | 3.86ab | 1.17a | 0.59c | 13.79abc | 16.29ab | 7.46bcde | 79.2 | 30.3ab |
| M9 | 5.0 | 0.64d | 0.77de | 2.89de | 0.57e | 0.63bc | 9.97d | 10.29bc | 5.65e | 75.0 | 31.4ab |
| | 6.5 | 0.73cd | 0.90e | 3.27cd | 0.69ed | 0.63bc | 13.29abcd | 10.76abc | 7.25bcde | 104.1 | 21.2cd |
| | 8 | 0.94ab | 1.39a | 4.28a | 1.12ab | 0.64bc | 15.95a | 17.34a | 9.00ab | 116.4 | 27.9bc |
| Interaction significance | | ** | ** | ** | ** | ** | ** | * | ** | NS | ** |

[z] Mean within columns and section with the same letter are not significantly different using Duncan's at MRT p≤0.05. *,**,*** or NS indicates treatment had a significant effect at P≤0.05 or P≤0.01 or P≤0.001 levels or had a non-significat effect, respectively.

Table 27.Multivariate correlation between soil pH and leaf nutrients of Honeycrisp apple on four rootstocks grown in minirhizotron system 2018.

| | pH | N | P | K | Ca | Mg | S | B | Zn | Cu | Mn | Na | Fe |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.0209 | 0.3479 | 0.6048 | 0.6048 | 0.4748 | -0.3162 | 0.3821 | 0.0477 | -0.0440 | 0.3479 | -0.3106 | -0.3498 |
| N | 0.0209 | 1.0000 | 0.8061 | 0.3557 | 0.3557 | 0.4382 | 0.7740 | -0.2137 | 0.2416 | 0.2625 | 0.4296 | -0.2133 | 0.2564 |
| P | 0.3479 | 0.8061 | 1.0000 | 0.5441 | 0.5441 | 0.5532 | 0.5357 | 0.0451 | 0.3099 | 0.2753 | 0.4110 | -0.2728 | 0.1394 |
| K | 0.6048 | 0.3557 | 0.5441 | 1.0000 | 1.0000 | 0.7129 | 0.1566 | 0.3389 | 0.3298 | 0.1346 | 0.6037 | 0.1127 | 0.1992 |
| Ca | 0.6048 | 0.3557 | 0.5441 | 1.0000 | 1.0000 | 0.7129 | 0.1566 | 0.3389 | 0.3298 | 0.1346 | 0.6037 | 0.1127 | 0.1992 |
| Mg | 0.4748 | 0.4382 | 0.5532 | 0.7129 | 0.7129 | 1.0000 | 0.1997 | -0.0747 | 0.2978 | 0.0340 | 0.3022 | -0.1856 | 0.2683 |
| S | -0.3162 | 0.7740 | 0.5357 | 0.1566 | 0.1566 | 0.1997 | 1.0000 | -0.3065 | 0.3362 | 0.2806 | 0.1822 | 0.1351 | 0.4608 |
| B | 0.3821 | -0.2137 | 0.0451 | 0.3389 | 0.3389 | -0.0747 | -0.3065 | 1.0000 | 0.1268 | 0.1715 | 0.1492 | -0.0785 | -0.1518 |
| Zn | 0.0477 | 0.2416 | 0.3099 | 0.3298 | 0.3298 | 0.2978 | 0.3362 | 0.1268 | 1.0000 | 0.4368 | 0.2455 | 0.0784 | 0.5191 |
| Cu | -0.0440 | 0.2625 | 0.2753 | 0.1346 | 0.1346 | 0.0340 | 0.2806 | 0.1715 | 0.4368 | 1.0000 | 0.1501 | -0.0940 | 0.2008 |
| Mn | 0.3479 | 0.4296 | 0.4110 | 0.6037 | 0.6037 | 0.3022 | 0.1822 | 0.1492 | 0.2455 | 0.1501 | 1.0000 | 0.1142 | 0.1105 |
| Na | -0.3106 | -0.2133 | -0.2728 | 0.1127 | 0.1127 | -0.1856 | 0.1351 | -0.0785 | 0.0784 | -0.0940 | 0.1142 | 1.0000 | 0.3613 |
| Fe | -0.3498 | 0.2564 | 0.1394 | 0.1992 | 0.1992 | 0.2683 | 0.4608 | -0.1518 | 0.5191 | 0.2008 | 0.1105 | 0.3613 | 1.0000 |

Table 29. Multivariate correlation between soil pH and root morphology parameters of Honeycrisp' from four rootstocks grown in minirhizotron system 2017- 2018

| ` | Depth | pH | DAP | Sum (Root Count) | Sum (Total Root Length) | Sum (Total Root Volume ^3) | Sum (Total Root Area ^2) | Mean (Average Root Diameter) | Mean (Average Root Area ^2) | Mean (Average Root Volume ^3) | Mean (Average Root Length) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Depth | 1.0000 | -0.0819 | 0.2289 | -0.0455 | -0.0519 | -0.0517 | -0.0730 | -0.2377 | -0.0517 | -0.0517 | -0.1195 |
| pH | -0.0819 | 1.0000 | -0.0238 | -0.2574 | -0.2720 | -0.0580 | -0.0329 | 0.0076 | -0.0580 | -0.0580 | -0.0146 |
| DAP | 0.2289 | -0.0238 | 1.0000 | 0.1608 | 0.0907 | -0.0141 | -0.0753 | -0.5412 | -0.0141 | -0.0141 | -0.3839 |
| Sum (Root Count) | -0.0455 | -0.2574 | 0.1608 | 1.0000 | 0.9791 | -0.0353 | -0.0238 | -0.3274 | -0.0353 | -0.0353 | -0.2461 |
| Sum (Total Root Length) | -0.0519 | -0.2720 | 0.0907 | 0.9791 | 1.0000 | 0.1565 | 0.0221 | -0.2874 | -0.2465 | -0.1905 | -0.1542 |
| Sum (Total Root Volume ^3) | -0.0517 | -0.0580 | -0.0141 | -0.0353 | 0.1565 | 1.0000 | 0.6471 | 0.5088 | 1.0000 | 1.0000 | 0.2913 |
| Sum (Total Root Area ^2) | -0.0730 | -0.0329 | -0.0753 | -0.0238 | 0.0221 | 0.6471 | 1.0000 | 0.0077 | 0.6471 | 0.6471 | 0.0413 |
| Mean(Average Root Diameter) | -0.2377 | 0.0076 | -0.5412 | -0.3274 | -0.2874 | 0.5088 | 0.0077 | 1.0000 | 0.8747 | 0.8778 | 0.4026 |
| Mean(Average Root Length) | -0.1195 | -0.0146 | -0.3839 | -0.2461 | -0.1542 | 0.2913 | 0.0413 | 0.4026 | 0.7069 | 0.5519 | 1.0000 |
| Mean(Average Root Area ^2) | -0.0517 | -0.0580 | -0.0141 | -0.0353 | -0.2465 | 1.0000 | 0.6471 | 0.8747 | 1.0000 | 1.0000 | 0.7069 |
| Mean(Average Root Volume ^3) | -0.0517 | -0.0580 | -0.0141 | -0.0353 | -0.1905 | 1.0000 | 0.6471 | 0.8778 | 1.0000 | 1.0000 | 0.5519 |

## 4. Discussion

The utilization of fruit bins as a Rhizobox was a perfect setup however the ground level should be maintained to make sure water level is not skewed or drained to certain down points. This was found to cause to sweep the soil from the rhizobox and expose top observation tubes to direct sunlight. This also created more moist conditions on one side of the rhizobox influencing more root's growth.

Since the fruit bins were ventilated from all four sides, it worth lining the inner of the fruit bins with a garden fabric before adding the soil to prevent spillage of water and soil while irrigation. The materials of the observation tubes were affected with high and low soil pH creating a foggy effect causing blurry images that sometimes were hard to trace fine roots. The material of the observation was reported to have an effect on root growth and development as reported by (Withington et al., 2003). Also, during the summertime, some roots on outer rhizobox exposed more to sunlight have a faster root turnover than the same replicate on inner rhizobox. This finding was also reported by (Rewald and Ephrath, 2013) when comparing CAB and acrylic observation tube effects on root browning.

**The root imager CID-602**

The root imager is an excellent image capturing device that allows 360° rotation capturing a perimeter of the tube in a single take. This allows faster and eliminates the need for image stitching. The root imager is also able to take a repeated image within minutes, hours or days when is programmed for repeated image capturing. However, great care must be taken while using it since it is not waterproof and need to be kept away from soil particle that can interfere with the mechanical moving parts.

**RootSnap image processing software.**

The software was released by the same developers who designed the root imager and hence it is compatible with the images produced by the CI-602 root imager. The software offers a user interface that employs a multi-touch LCD screen making root tracing fast and accurate. However, great attention needs to be taken while tracing small and fine roots where tracing tools must match the root diameter to ensure accurate reading and calculation of the root parameters. The clarity of the image and the resolution is important in tracing roots withing mixed soil particles that might be misidentified with root segments. It also very important to distinguish fine root within the soil with scratches on the inner side of the observation tube is not clear.

The output of the RooSnap® includes physical Size measurements of root parameter such as root count, total root, length total, root volume, total root area, average root diameter, average root length, average root area, average root volume, and estimated root area. Although the great advantages of using computer software programs to measure root's parameters facilitate faster and easy quantitative data generation, disadvantages can be found as well (Judd et al., 2015).

**Root morphology**

The non-significant effect of the rootstocks on the root morphology could be explained by many theories. Tube installation knows to causes soil disturbance and has the potential to create artifacts and voids in subsequent root data and analysis. A recommend a waiting period between tube installation and an image collection of 6–12 months to allow roots growth and distribution within the space around the tubes and to permit nutrients to return to pre-disturbance levels (Johnson et al., 2001). In this study,

an immediate image collection was conducted right after installation. This could lead to similarity within rootstocks in root development and appearance in observation windows.

Since two of the tested rootstock (G.890 and G.214) were sharing the same parents could explain similar root patterns. However, G.890 is a semi-dwarf rootstock while G.214 is in the dwarf rootstock class. This also could explain the similar root growth pattern between rootstock G.214 and M.9. Although rootstock G.41 parents are different than the other geneva rootstocks tested in this study, it also shows similar root morphology. This could be related to the strong effect of the soil pH in influencing root's growth and developments.

The size and age of the tree also influence how their lateral root distributes within the soil profile. As reported by (Stokes et al., 1995) in regards to how the loading force and stability shape the root system. Since all trees were small and young, similar root morphology was expected.

This finding can be linked to finding by (Hughes and Gandar, 1993) who concluded that the root distribution of young apple trees differs from older trees. The shape of root pattern of young apple trees follows and bowl-shaped where roots are centered near the stem, while the older tree has more layered root structure distributed away from the trunk (De Silva et al., 1999). Other findings on M.9 rootstock reported the several growth peaks during the single growing season. This could be compared to result by (Psarras et al. 2000, Ma et al., 2013, and Wang et al.1997) who supported root growth peaks on apple rootstocks.

Another explanation of this similarity within rootstocks could be due to the

uniformity of soil structure. This finding is consistent with that of (Fan and Yang, 2008) who found the effect of soil structure (texture and particle size) on apple root architecture. Reporting that well-distributed lateral roots were based on soil particles and texture. Hence the soil used in all rhizoboxes was identical in terms of soil's texture and organic matters.

However, a highly significant difference was found within soil pH treatment was predicted. This finding broadly supports the work of other studies in this area linking root's growth with the effect of root architecture on the nutrient uptake from the rhizosphere (Barber and Silberbush 1984, Itoh and Barber 1983). Since the pH level affects the availability of nutrients, root growth would be highly affected by varying soil pH treatments (Asao 2012).

Statistical data of leaf nutrients analysis shows a higher concentration of phosphorus, nitrogen, calcium, magnesium, boron, zinc, copper, and manganese at pH 8.0 treatment. However, the higher rate of root morphological parameters was at low pH treatments. Interpretation of soil analysis allows fertilizer recommendation, however, it is not necessarily would evaluate the efficiency or sufficiency of nutrient uptake in apple growth and tree productivity (Ge et al. 2018).

## 5. Conclusion

The study was conducted in two years to monitor four apple rootstocks under soil pH treatments to evaluate the growth and distribution of the root system within the soil profile. This was a successful attempt to optimized a portable, refurbish-able and easy to use minirhizotron system for fruit trees in general and apple trees in precise. The 360° revolving root scanner facilitated faster images taking at varying resolution levels. However, to reduce variability, soil compaction and settling must be maintained before transplanting to avoid gaps and void in between observation tubes and surrounding soil. Since soil cracks found to capture more moisture and thus influencing root growth within, a soil selection that tolerates cracking would be recommended in for minirhizotron setup.

Also, the rhizobox could be improved by incorporating temperature and moister sensors to detect changes associated with those conditions. Evenly watering and maintaining uniform moister condition through the soil profile in the rhizobox would eliminate soil drying and thus root's retard growth due to drought.

Conducting this study on other genetically diverse apple rootstocks will improve root's distribution understanding within the soil profile. Also, a long term monitoring of the root system under different fertilizers scheme or soil type would provide a clear understanding of the response of root system of apple rootstock tp those variables. Finally, a weatherproof root's scanner would be ideal for field data collection in open field setup.

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# APPENDIX

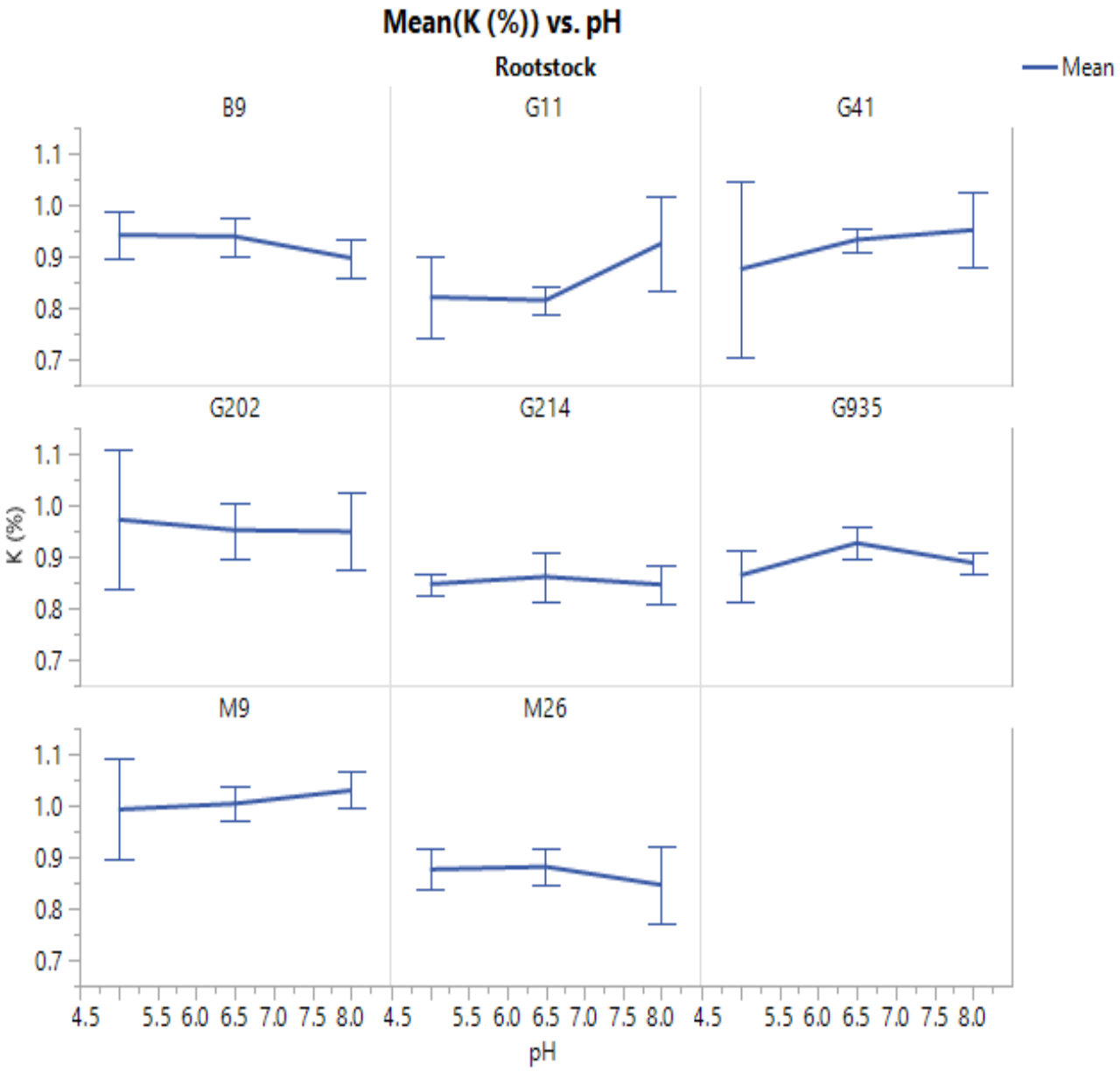


Figure 19. Interaction of soil pH and eight rootstocks on fruit potassium concentration of Honeycrisp' grown under varying soil pH levels at Ithaca

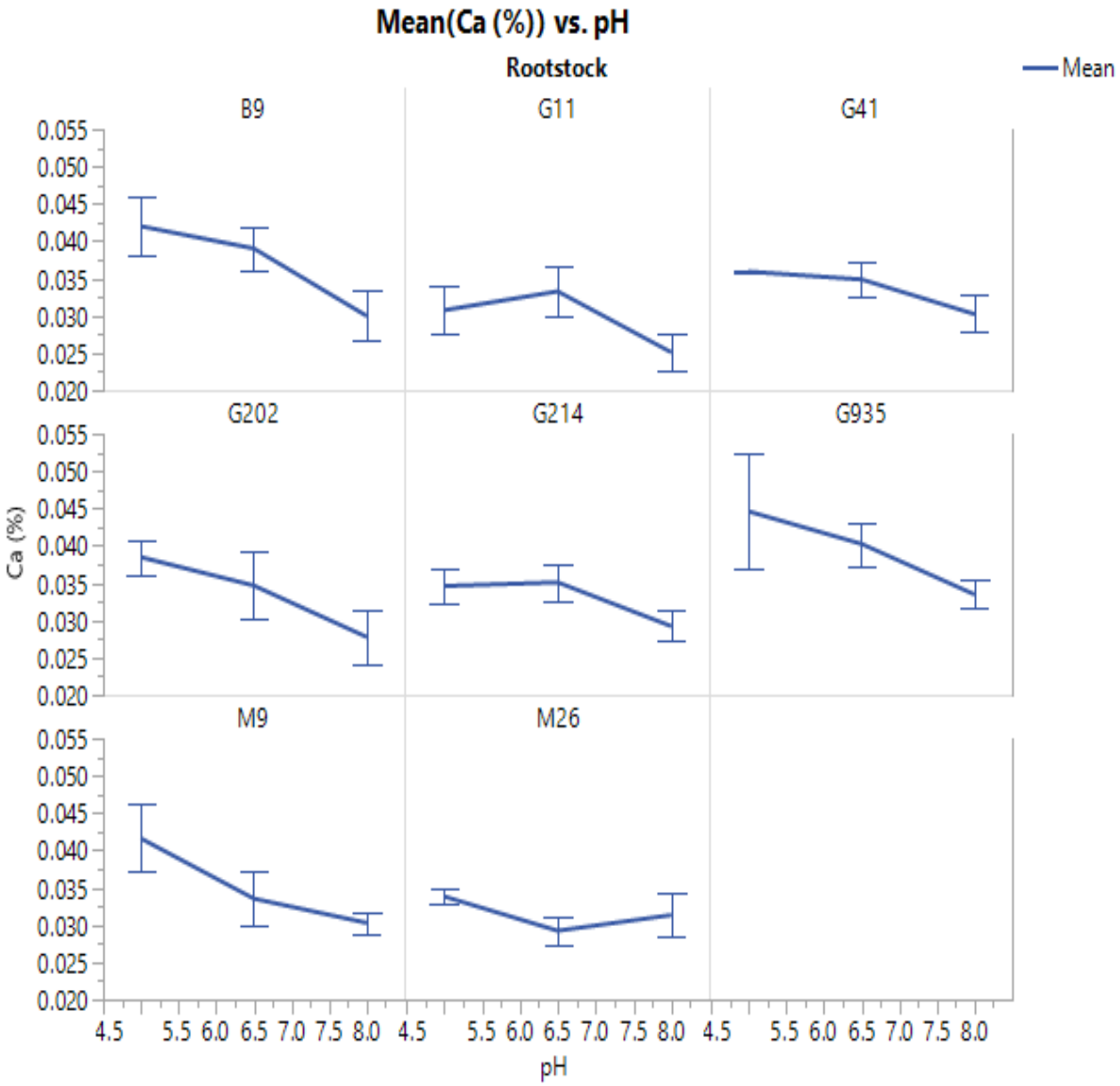


Figure 20. Interaction of soil pH and eight rootstocks on fruit calcium concentration of Honeycrisp' grown under varying soil pH levels at Ithaca

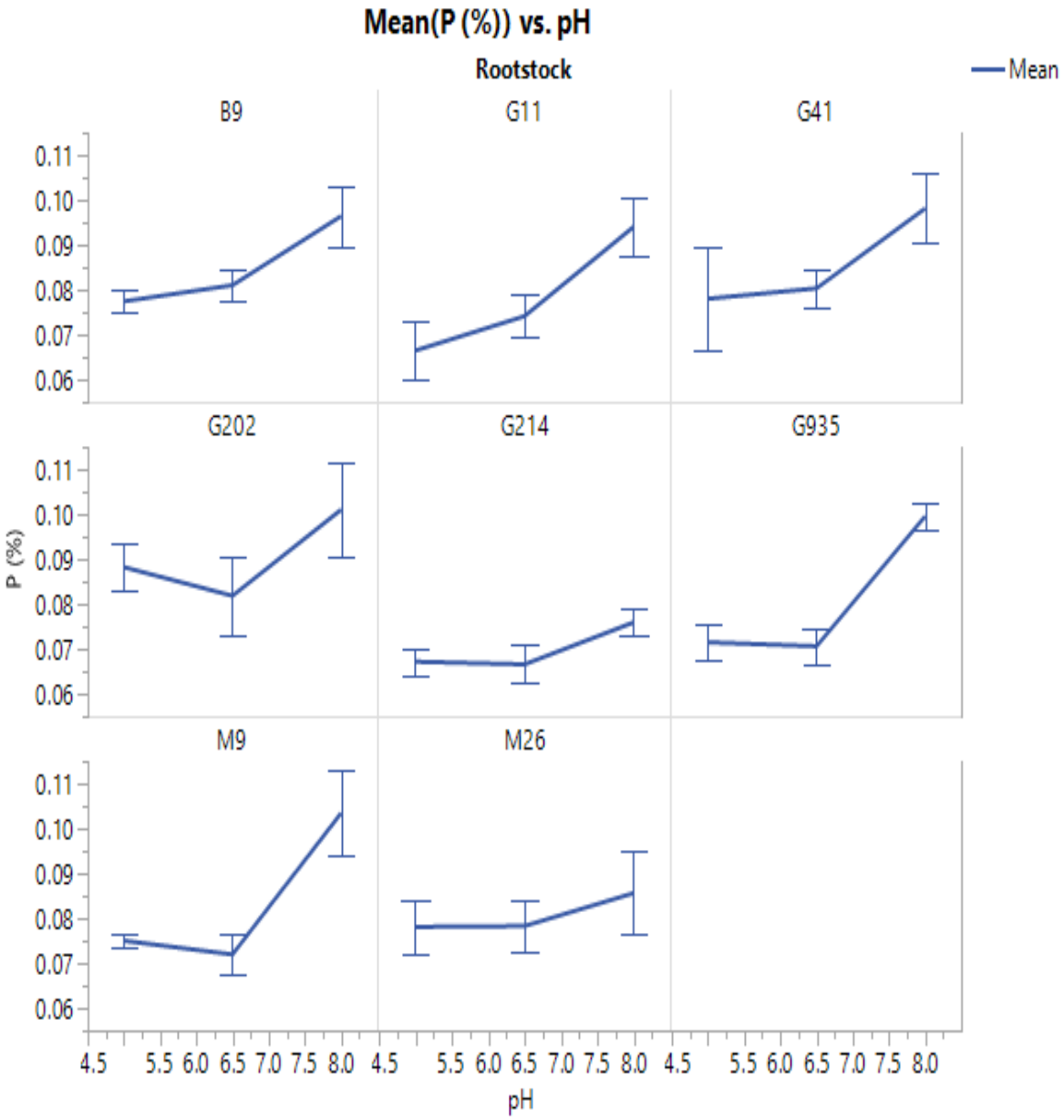


Figure 21. Interaction of soil pH and eight rootstocks on fruit phosphorus concentration of Honeycrisp' grown under varying soil pH levels at Ithaca.

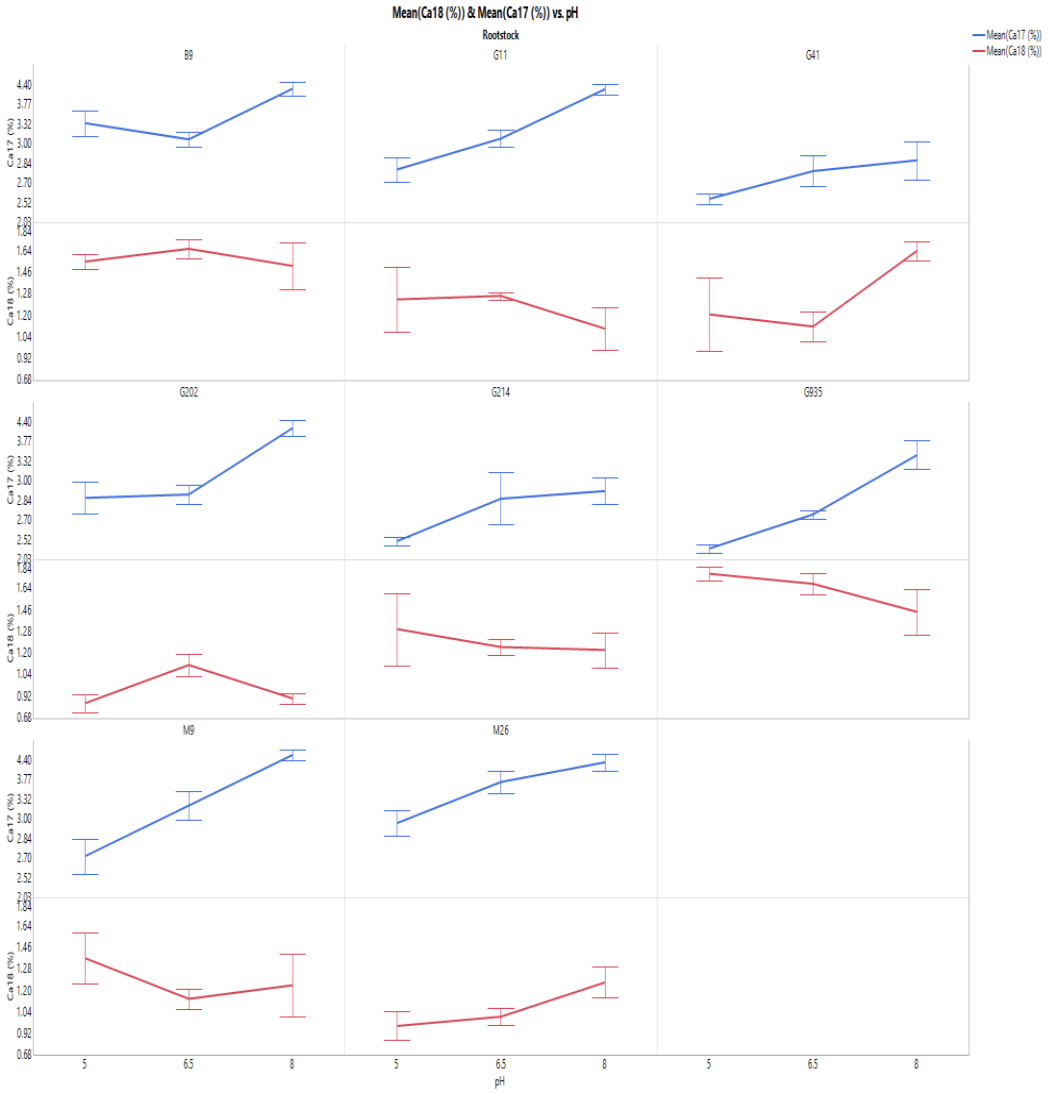


Figure 22. Comparison of means of leaf calcium contents from 2017 and 2018 by rootstock.

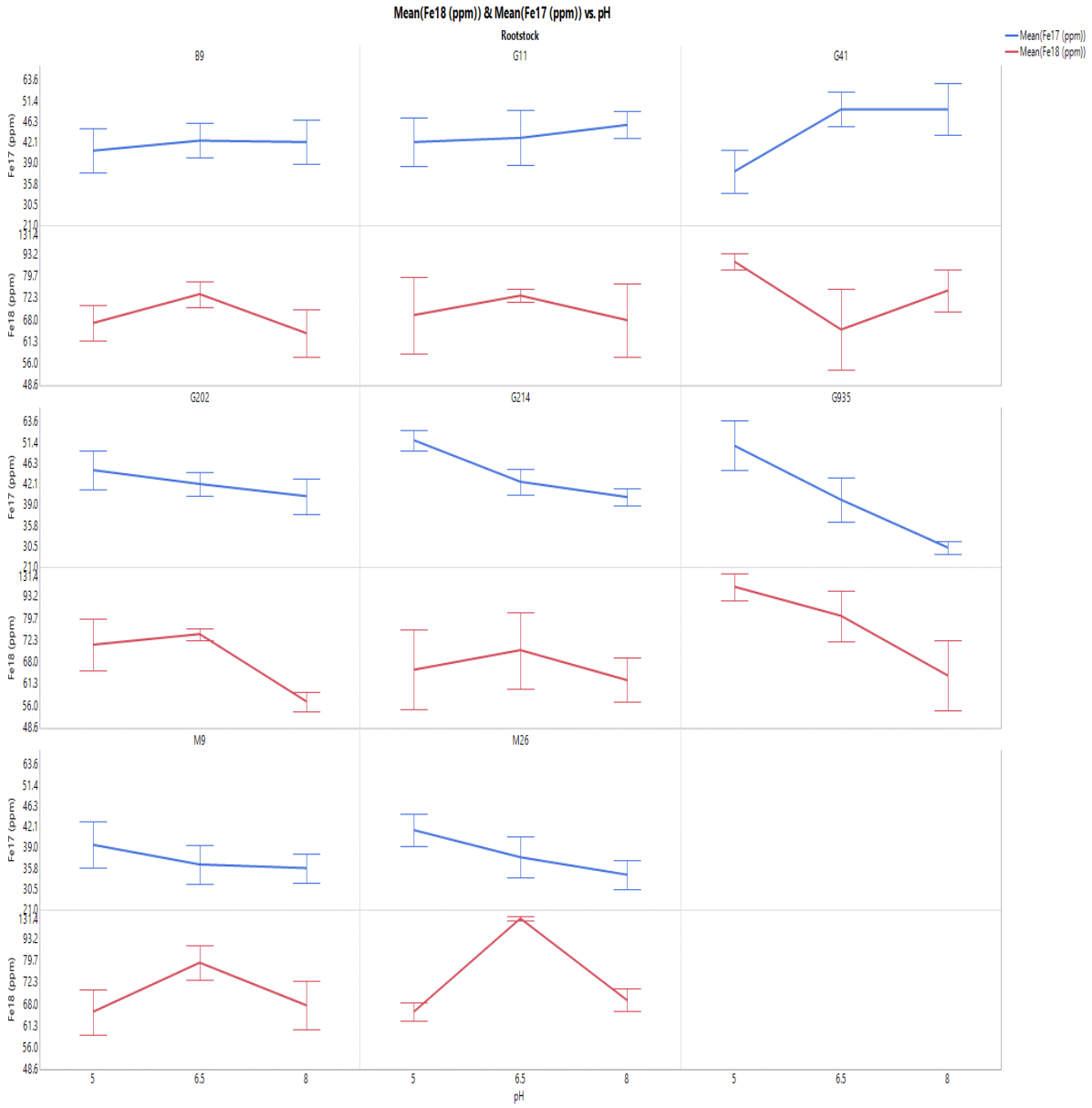


Figure 23. Comparison of means of leaf iron contents from 2017 and 2018 by rootstock.

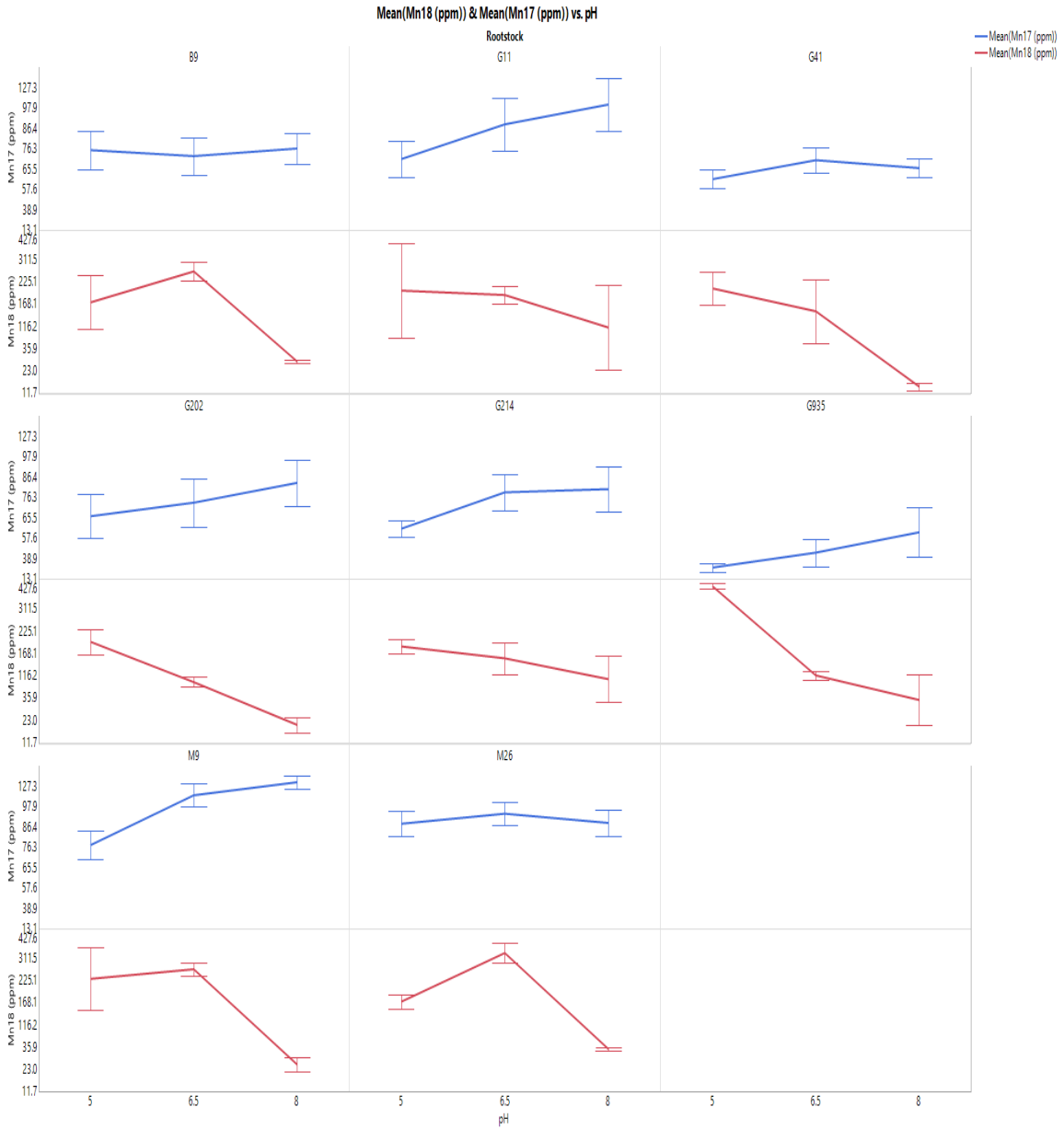


Figure 24. Comparison of means of leaf manganese contents from 2017 and 2018 by rootstock.

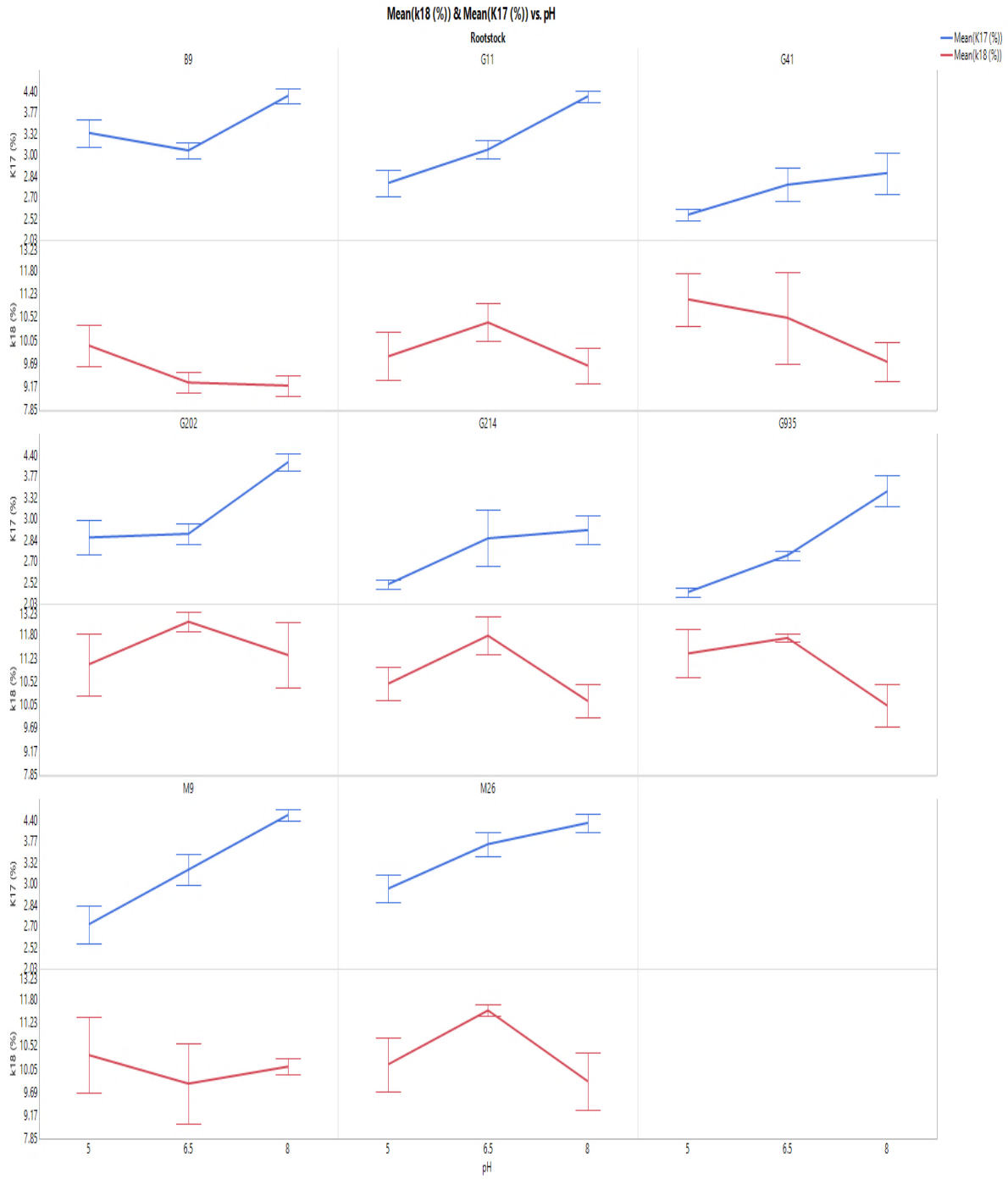


Figure 25. Comparison of means of leaf potassium contents from 2017 and 2018 by rootstock.

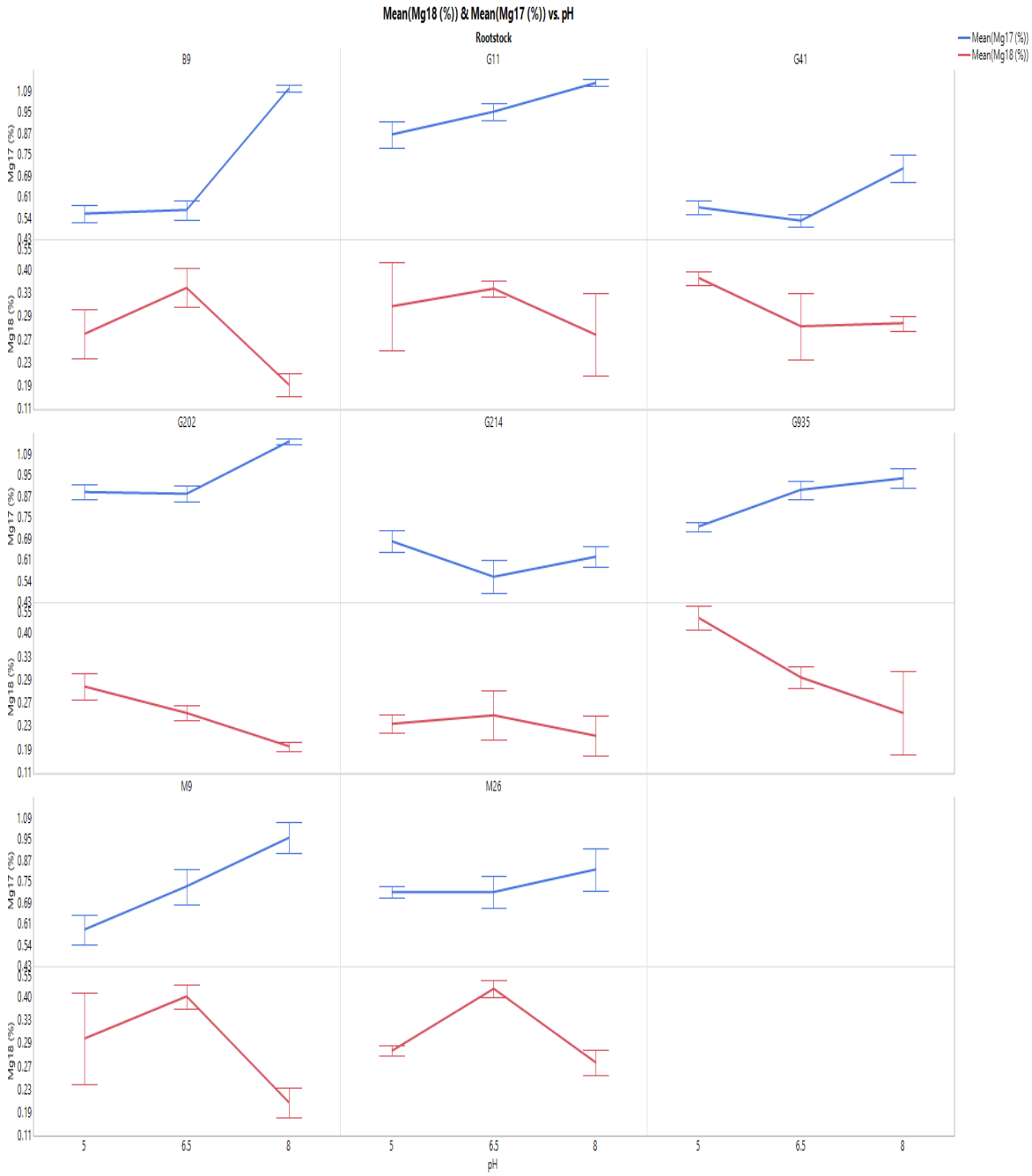


Figure 26. Comparison of means of leaf magnesium contents from 2017 and 2018 by rootstock.

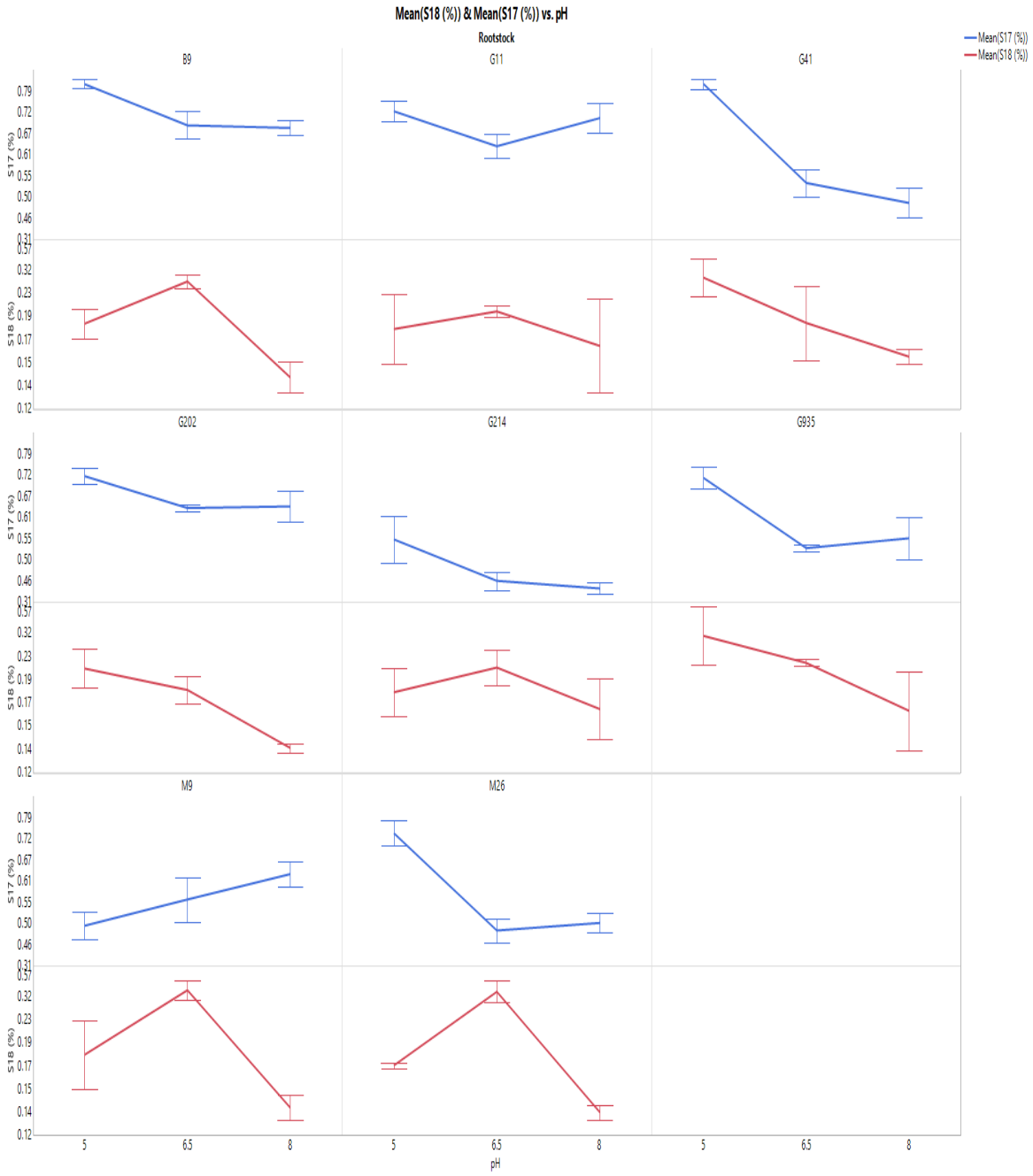


Figure 27. Comparison of means of leaf sulfur contents from 2017 and 2018 by rootstock.

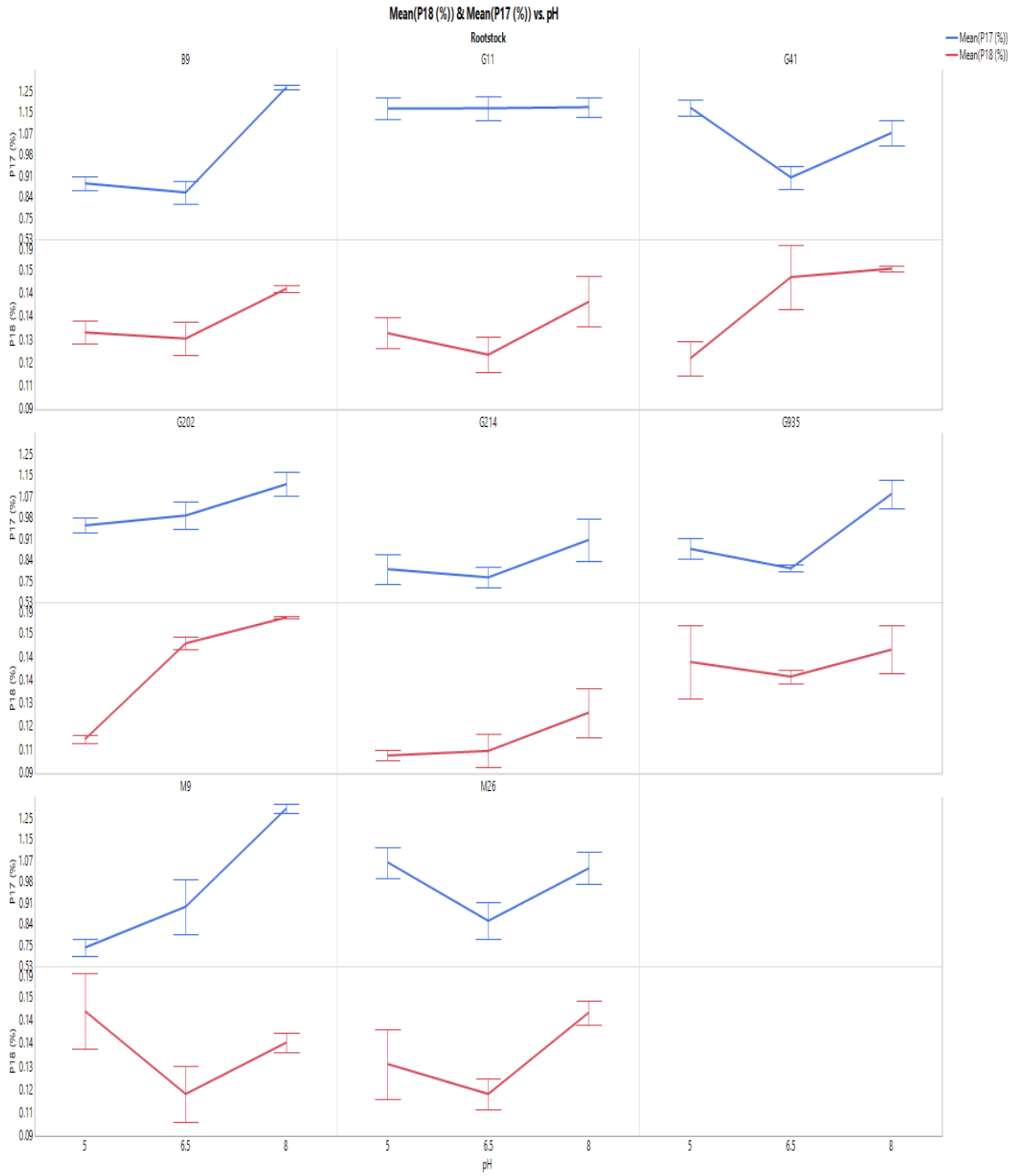


Figure 28. Comparison of means of leaf phosphorus contents from 2017 and 2018 by rootstock.

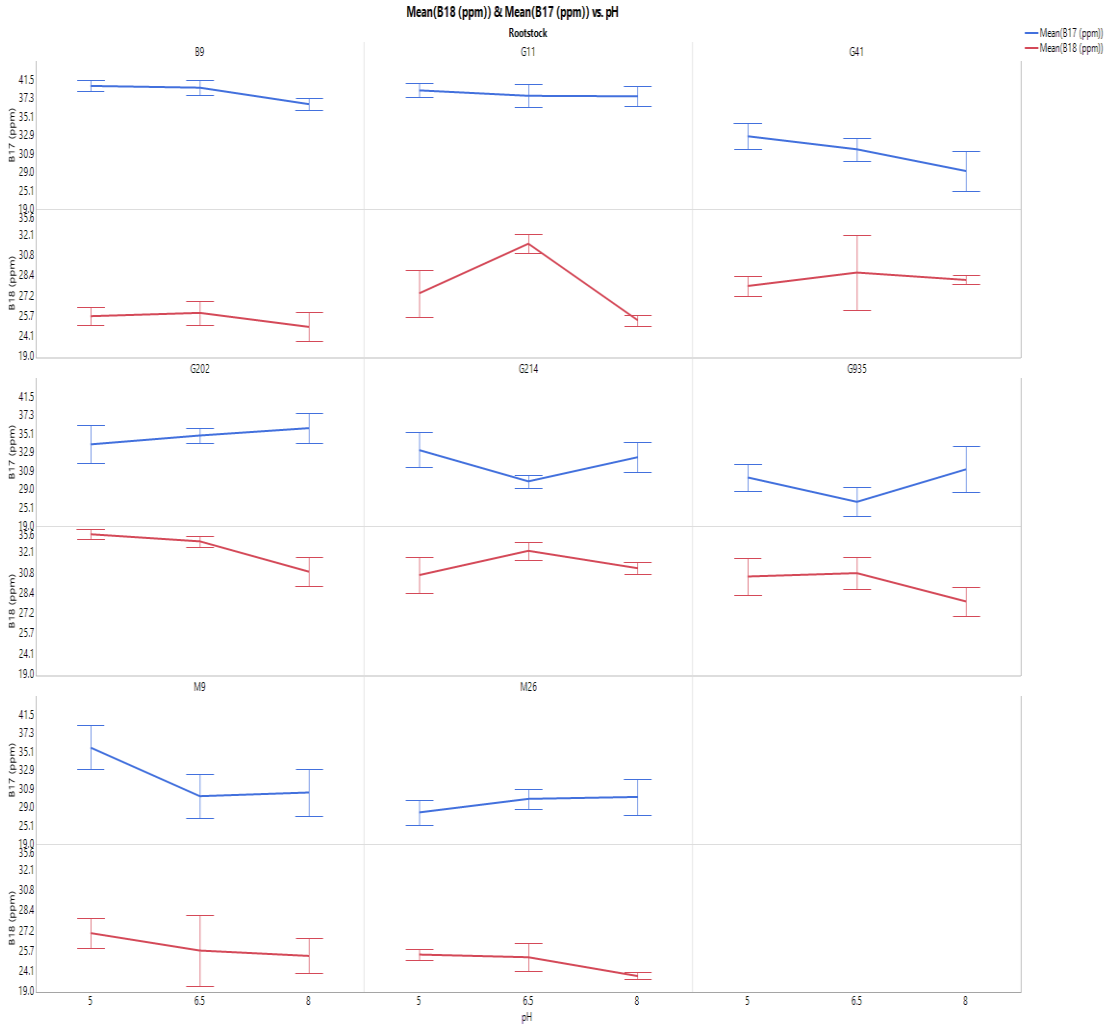


Figure 29. Comparison of means of leaf boron contents from 2017 and 2018 by rootstock.

## Rootstock=B9

Table 30.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from B.9 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.5644* | 0.0522 | -0.0557 | -0.4257* | -0.2938 | 0.5504* | -0.1810 | -0.5040* | 0.4369 | -0.2193 |
| Fruit weight (g) | 0.5644 * | 1.0000 | 0.1360 | -0.0215 | -0.3100 | -0.1384 | 0.2328 | -0.4500* | -0.4063 | 0.1995 | -0.4555* |
| Red skin % | 0.0522 | 0.1360 | 1.0000 | 0.0630 | -0.0673 | -0.2025 | 0.1854 | -0.0347 | -0.2917 | 0.1894 | -0.0273 |
| TSS (°Bx) | -0.0557 | -0.0215 | 0.0630 | 1.0000 | 0.1738 | -0.4010 | -0.0720 | 0.2013 | 0.0292 | -0.2405 | 0.1112 |
| Firmness (kg) | -0.4257* | -0.3100 | -0.0673 | 0.1738 | 1.0000 | 0.4809* | -0.1835 | 0.5411* | 0.5589* | -0.3470 | 0.5541* |
| Yield (fruit) | -0.2938 | -0.1384 | -0.2025 | -0.4010* | 0.4809 | 1.0000 | -0.1336 | 0.0260 | 0.3217 | -0.1436 | 0.2293 |
| P | 0.5504* | 0.2328 | 0.1854 | -0.0720 | -0.1835 | -0.1336 | 1.0000 | 0.2461 | -0.5046* | 0.6998* | 0.2400 |
| K | -0.1810 | -0.4500* | -0.0347 | 0.2013 | 0.5411 | 0.0260 | 0.2461 | 1.0000 | 0.1501 | -0.1839 | 0.7121* |
| Ca | -0.5040* | -0.4063* | -0.2917 | 0.0292 | 0.5589 | 0.3217 | -0.5046* | 0.1501 | 1.0000 | -0.4844* | 0.1960 |
| Mg | 0.4369* | 0.1995 | 0.1894 | -0.2405 | -0.3470 | -0.1436 | 0.6998* | -0.1839 | -0.4844* | 1.0000 | 0.0417 |
| S | -0.2193 | -0.4555* | -0.0273 | 0.1112 | 0.5541 | 0.2293 | 0.2400 | 0.7121* | 0.1960 | 0.0417 | 1.0000 |

## Rootstock=B9

Table 31. Multivariate correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from B.9 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.5644 | 0.0522 | -0.0557 | -0.4257 | -0.2938 | -0.2788 | -0.1244 | -0.3338 | -0.5663 | -0.0853 |
| Fruit weight (g) | 0.5644 | 1.0000 | 0.1360 | -0.0215 | -0.3100 | -0.1384 | -0.0370 | 0.1375 | -0.3327 | -0.5481 | -0.0340 |
| Red skin % | 0.0522 | 0.1360 | 1.0000 | 0.0630 | -0.0673 | -0.2025 | -0.1632 | -0.1326 | 0.0899 | 0.0071 | -0.1222 |
| TSS (°Bx) | -0.0557 | -0.0215 | 0.0630 | 1.0000 | 0.1738 | -0.4010 | -0.0853 | -0.4789 | -0.4698 | -0.1001 | 0.2703 |
| Firmness (kg) | -0.4257 | -0.3100 | -0.0673 | 0.1738 | 1.0000 | 0.4809 | -0.0291 | 0.1505 | 0.0160 | 0.5400 | 0.6074 |
| Yield (fruit) | -0.2938 | -0.1384 | -0.2025 | -0.4010 | 0.4809 | 1.0000 | -0.0948 | 0.4602 | 0.2974 | 0.3234 | -0.0169 |
| B | -0.2788 | -0.0370 | -0.1632 | -0.0853 | -0.0291 | -0.0948 | 1.0000 | 0.1089 | 0.1978 | 0.0158 | 0.0266 |
| Zn | -0.1244 | 0.1375 | -0.1326 | -0.4789 | 0.1505 | 0.4602 | 0.1089 | 1.0000 | 0.2497 | 0.3317 | 0.3270 |
| Fe | -0.3338 | -0.3327 | 0.0899 | -0.4698 | 0.0160 | 0.2974 | 0.1978 | 0.2497 | 1.0000 | 0.6592 | -0.1075 |
| Mn | -0.5663 | -0.5481 | 0.0071 | -0.1001 | 0.5400 | 0.3234 | 0.0158 | 0.3317 | 0.6592 | 1.0000 | 0.3821 |
| Cu | -0.0853 | -0.0340 | -0.1222 | 0.2703 | 0.6074 | -0.0169 | 0.0266 | 0.3270 | -0.1075 | 0.3821 | 1.0000 |

## Rootstock=B9

Table 32.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratios of Honeycrisp' from B.9 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.5644 | 0.0522 | -0.0557 | -0.4257 | -0.2938 | 0.5319 | 0.4713 | 0.5893 | 0.6273 | -0.1806 | -0.1465 |
| Fruit weight (g) | 0.5644 | 1.0000 | 0.1360 | -0.0215 | -0.3100 | -0.1384 | 0.5504 | 0.2503 | 0.4145 | 0.3983 | -0.4498 | -0.4386 |
| Red skin % | 0.0522 | 0.1360 | 1.0000 | 0.0630 | -0.0673 | -0.2025 | -0.0405 | 0.3624 | 0.3940 | 0.3875 | -0.0345 | -0.0192 |
| TSS (°Bx) | -0.0557 | -0.0215 | 0.0630 | 1.0000 | 0.1738 | -0.4010 | 0.1295 | 0.0509 | -0.0686 | -0.0401 | 0.2012 | 0.1838 |
| Firmness (kg) | -0.4257 | -0.3100 | -0.0673 | 0.1738 | 1.0000 | 0.4809 | -0.5496 | -0.3344 | -0.5423 | -0.4580 | 0.5408 | 0.5185 |
| Yield (fruit) | -0.2938 | -0.1384 | -0.2025 | -0.4010 | 0.4809 | 1.0000 | -0.3651 | -0.2807 | -0.3151 | -0.2648 | 0.0257 | 0.0142 |
| Bitter pit | 0.5319 | 0.5504 | -0.0405 | 0.1295 | -0.5496 | -0.3651 | 1.0000 | 0.2773 | 0.4733 | 0.4201 | -0.2832 | -0.2593 |
| K/Ca | 0.4713 | 0.2503 | 0.3624 | 0.0509 | -0.3344 | -0.2807 | 0.2773 | 1.0000 | 0.8633 | 0.9050 | 0.1861 | 0.2278 |
| Mg/Ca | 0.5893 | 0.4145 | 0.3940 | -0.0686 | -0.5423 | -0.3151 | 0.4733 | 0.8633 | 1.0000 | 0.9390 | -0.2258 | -0.1655 |
| P/Ca | 0.6273 | 0.3983 | 0.3875 | -0.0401 | -0.4580 | -0.2648 | 0.4201 | 0.9050 | 0.9390 | 1.0000 | -0.0415 | 0.0148 |
| K+Mg/Ca | -0.1806 | -0.4498 | -0.0345 | 0.2012 | 0.5408 | 0.0257 | -0.2832 | 0.1861 | -0.2258 | -0.0415 | 1.0000 | 0.9967 |
| K+Mg+P/Ca | -0.1465 | -0.4386 | -0.0192 | 0.1838 | 0.5185 | 0.0142 | -0.2593 | 0.2278 | -0.1655 | 0.0148 | 0.9967 | 1.0000 |

## Multivariate correlations
## Rootstock=G11

Table 33.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from G.11 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2032 | -0.3907 | -0.0594 | -0.0865 | -0.2472 | 0.5744 | 0.2735 | -0.1822 | 0.5225 | -0.2434 |
| Fruit weight (g) | 0.2032 | 1.0000 | 0.3639 | -0.2012 | -0.5185 | -0.0541 | -0.0463 | -0.3537 | -0.2883 | 0.5814 | -0.4355 |
| Red skin % | -0.3907 | 0.3639 | 1.0000 | -0.5458 | -0.0516 | 0.2472 | -0.1375 | -0.0972 | 0.0329 | 0.1236 | -0.0072 |
| TSS (°Bx) | -0.0594 | -0.2012 | -0.5458 | 1.0000 | -0.1872 | -0.1983 | -0.0449 | -0.0473 | -0.1860 | -0.3634 | 0.2387 |
| Firmness (kg) | -0.0865 | -0.5185 | -0.0516 | -0.1872 | 1.0000 | -0.0284 | 0.0979 | 0.2761 | -0.2021 | -0.1984 | 0.0141 |
| Yield (fruit) | -0.2472 | -0.0541 | 0.2472 | -0.1983 | -0.0284 | 1.0000 | -0.2379 | -0.1354 | 0.4718 | -0.2567 | -0.0144 |
| P | 0.5744 | -0.0463 | -0.1375 | -0.0449 | 0.0979 | -0.2379 | 1.0000 | 0.7254 | -0.2359 | 0.4892 | 0.2208 |
| K | 0.2735 | -0.3537 | -0.0972 | -0.0473 | 0.2761 | -0.1354 | 0.7254 | 1.0000 | -0.1466 | 0.1664 | 0.7208 |
| Ca | -0.1822 | -0.2883 | 0.0329 | -0.1860 | -0.2021 | 0.4718 | -0.2359 | -0.1466 | 1.0000 | -0.2689 | -0.0029 |
| Mg | 0.5225 | 0.5814 | 0.1236 | -0.3634 | -0.1984 | -0.2567 | 0.4892 | 0.1664 | -0.2689 | 1.0000 | -0.3026 |
| S | -0.2434 | -0.4355 | -0.0072 | 0.2387 | 0.0141 | -0.0144 | 0.2208 | 0.7208 | -0.0029 | -0.3026 | 1.0000 |

## Rootstock=G11

Table 34.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from G.11 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2032 | -0.3907 | -0.0594 | -0.0865 | -0.2472 | -0.5141 | -0.1984 | -0.0973 | -0.5222 | 0.3468 |
| Fruit weight (g) | 0.2032 | 1.0000 | 0.3639 | -0.2012 | -0.5185 | -0.0541 | -0.0312 | -0.0194 | -0.2801 | 0.1340 | -0.0087 |
| Red skin % | -0.3907 | 0.3639 | 1.0000 | -0.5458 | -0.0516 | 0.2472 | 0.2649 | 0.1825 | 0.1605 | 0.4356 | 0.0494 |
| TSS (°Bx) | -0.0594 | -0.2012 | -0.5458 | 1.0000 | -0.1872 | -0.1983 | -0.0181 | -0.0333 | -0.2932 | -0.5550 | -0.2322 |
| Firmness (kg) | -0.0865 | -0.5185 | -0.0516 | -0.1872 | 1.0000 | -0.0284 | 0.0582 | -0.3093 | -0.1844 | -0.0906 | 0.0085 |
| Yield (fruit) | -0.2472 | -0.0541 | 0.2472 | -0.1983 | -0.0284 | 1.0000 | -0.2668 | 0.5055 | 0.5808 | 0.5390 | 0.0539 |
| B | -0.5141 | -0.0312 | 0.2649 | -0.0181 | 0.0582 | -0.2668 | 1.0000 | -0.0929 | -0.3547 | 0.0069 | -0.2814 |
| Zn | -0.1984 | -0.0194 | 0.1825 | -0.0333 | -0.3093 | 0.5055 | -0.0929 | 1.0000 | 0.5987 | 0.4261 | 0.2548 |
| Fe | -0.0973 | -0.2801 | 0.1605 | -0.2932 | -0.1844 | 0.5808 | -0.3547 | 0.5987 | 1.0000 | 0.4095 | 0.3156 |
| Mn | -0.5222 | 0.1340 | 0.4356 | -0.5550 | -0.0906 | 0.5390 | 0.0069 | 0.4261 | 0.4095 | 1.0000 | -0.1290 |
| Cu | 0.3468 | -0.0087 | 0.0494 | -0.2322 | 0.0085 | 0.0539 | -0.2814 | 0.2548 | 0.3156 | -0.1290 | 1.0000 |

## Rootstock=G11

Table 35.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from G.11 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2032 | -0.3907 | -0.0594 | -0.0865 | -0.2472 | 0.3104 | 0.3728 | 0.4647 | 0.5244 | 0.2738 | 0.3191 |
| Fruit weight (g) | 0.2032 | 1.0000 | 0.3639 | -0.2012 | -0.5185 | -0.0541 | 0.1983 | 0.0944 | 0.5524 | 0.1878 | -0.3533 | -0.2891 |
| Red skin % | -0.3907 | 0.3639 | 1.0000 | -0.5458 | -0.0516 | 0.2472 | -0.2191 | -0.1281 | 0.0109 | -0.1248 | -0.0971 | -0.0831 |
| TSS (°Bx) | -0.0594 | -0.2012 | -0.5458 | 1.0000 | -0.1872 | -0.1983 | 0.0616 | 0.1632 | -0.0456 | 0.0861 | -0.0474 | -0.0818 |
| Firmness (kg) | -0.0865 | -0.5185 | -0.0516 | -0.1872 | 1.0000 | -0.0284 | -0.3119 | 0.2848 | -0.0314 | 0.1443 | 0.2761 | 0.2509 |
| Yield (fruit) | -0.2472 | -0.0541 | 0.2472 | -0.1983 | -0.0284 | 1.0000 | -0.2967 | -0.4285 | -0.4100 | -0.4067 | -0.1357 | -0.1579 |
| Bitter pit | 0.3104 | 0.1983 | -0.2191 | 0.0616 | -0.3119 | -0.2967 | 1.0000 | 0.1761 | 0.3919 | 0.4051 | -0.0332 | 0.0063 |
| K/Ca | 0.3728 | 0.0944 | -0.1281 | 0.1632 | 0.2848 | -0.4285 | 0.1761 | 1.0000 | 0.7746 | 0.9115 | 0.5451 | 0.5686 |
| Mg/Ca | 0.4647 | 0.5524 | 0.0109 | -0.0456 | -0.0314 | -0.4100 | 0.3919 | 0.7746 | 1.0000 | 0.8399 | 0.1089 | 0.1807 |
| P/Ca | 0.5244 | 0.1878 | -0.1248 | 0.0861 | 0.1443 | -0.4067 | 0.4051 | 0.9115 | 0.8399 | 1.0000 | 0.4365 | 0.4779 |
| K+Mg/Ca | 0.2738 | -0.3533 | -0.0971 | -0.0474 | 0.2761 | -0.1357 | -0.0332 | 0.5451 | 0.1089 | 0.4365 | 1.0000 | 0.9954 |
| K+Mg+P/Ca | 0.3191 | -0.2891 | -0.0831 | -0.0818 | 0.2509 | -0.1579 | 0.0063 | 0.5686 | 0.1807 | 0.4779 | 0.9954 | 1.0000 |

## Multivariate correlations
## Rootstock=G41

Table 36.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from G.41 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.5221 | 0.4494 | -0.0105 | -0.1518 | -0.0140 | 0.3974 | 0.1946 | -0.2536 | 0.5046 | -0.3160 |
| Fruit weight (g) | 0.5221 | 1.0000 | 0.6373 | -0.3356 | -0.0140 | 0.3282 | 0.1084 | 0.0195 | -0.2586 | 0.2698 | -0.0563 |
| Red skin % | 0.4494 | 0.6373 | 1.0000 | -0.0606 | 0.0551 | -0.1262 | 0.0533 | 0.1288 | -0.1433 | 0.0104 | 0.1397 |
| TSS (°Bx) | -0.0105 | -0.3356 | -0.0606 | 1.0000 | 0.4767 | -0.2944 | -0.4490 | -0.3766 | 0.4062 | -0.1297 | -0.3443 |
| Firmness (kg) | -0.1518 | -0.0140 | 0.0551 | 0.4767 | 1.0000 | 0.3010 | -0.3415 | -0.3483 | 0.3070 | -0.0613 | -0.4582 |
| Yield (fruit) | -0.0140 | 0.3282 | -0.1262 | -0.2944 | 0.3010 | 1.0000 | 0.0359 | 0.0139 | 0.0045 | 0.0895 | -0.2783 |
| P | 0.3974 | 0.1084 | 0.0533 | -0.4490 | -0.3415 | 0.0359 | 1.0000 | 0.6727 | -0.5282 | 0.6867 | -0.0742 |
| K | 0.1946 | 0.0195 | 0.1288 | -0.3766 | -0.3483 | 0.0139 | 0.6727 | 1.0000 | -0.1408 | 0.1508 | 0.3348 |
| Ca | -0.2536 | -0.2586 | -0.1433 | 0.4062 | 0.3070 | 0.0045 | -0.5282 | -0.1408 | 1.0000 | -0.2723 | -0.1202 |
| Mg | 0.5046 | 0.2698 | 0.0104 | -0.1297 | -0.0613 | 0.0895 | 0.6867 | 0.1508 | -0.2723 | 1.0000 | -0.4827 |
| S | -0.3160 | -0.0563 | 0.1397 | -0.3443 | -0.4582 | -0.2783 | -0.0742 | 0.3348 | -0.1202 | -0.4827 | 1.0000 |

## Rootstock=G41

Table 37.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from G.41 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.5221 | 0.4494 | -0.0105 | -0.1518 | -0.0140 | -0.2054 | -0.0805 | -0.0970 | -0.7103 | -0.1668 |
| Fruit weight (g) | 0.5221 | 1.0000 | 0.6373 | -0.3356 | -0.0140 | 0.3282 | -0.3461 | -0.1701 | 0.0532 | -0.4395 | -0.4259 |
| Red skin % | 0.4494 | 0.6373 | 1.0000 | -0.0606 | 0.0551 | -0.1262 | 0.0213 | -0.2036 | -0.2117 | -0.2539 | -0.2530 |
| TSS (°Bx) | -0.0105 | -0.3356 | -0.0606 | 1.0000 | 0.4767 | -0.2944 | 0.4210 | 0.0973 | 0.0570 | -0.0243 | 0.3826 |
| Firmness (kg) | -0.1518 | -0.0140 | 0.0551 | 0.4767 | 1.0000 | 0.3010 | 0.0384 | 0.3783 | -0.0103 | -0.1561 | 0.5162 |
| Yield (fruit) | -0.0140 | 0.3282 | -0.1262 | -0.2944 | 0.3010 | 1.0000 | -0.2386 | 0.5223 | 0.3016 | -0.1700 | 0.2587 |
| B | -0.2054 | -0.3461 | 0.0213 | 0.4210 | 0.0384 | -0.2386 | 1.0000 | -0.0957 | -0.0616 | 0.0255 | 0.3550 |
| Zn | -0.0805 | -0.1701 | -0.2036 | 0.0973 | 0.3783 | 0.5223 | -0.0957 | 1.0000 | -0.1935 | 0.0490 | 0.4736 |
| Fe | -0.0970 | 0.0532 | -0.2117 | 0.0570 | -0.0103 | 0.3016 | -0.0616 | -0.1935 | 1.0000 | -0.1117 | -0.0302 |
| Mn | -0.7103 | -0.4395 | -0.2539 | -0.0243 | -0.1561 | -0.1700 | 0.0255 | 0.0490 | -0.1117 | 1.0000 | -0.1628 |
| Cu | -0.1668 | -0.4259 | -0.2530 | 0.3826 | 0.5162 | 0.2587 | 0.3550 | 0.4736 | -0.0302 | -0.1628 | 1.0000 |

## Rootstock=G41

Table 38.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from G.41 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.5221 | 0.4494 | -0.0105 | -0.1518 | -0.0140 | -0.0730 | 0.3561 | 0.5150 | 0.4281 | 0.1948 | 0.2429 |
| Fruit weight (g) | 0.5221 | 1.0000 | 0.6373 | -0.3356 | -0.0140 | 0.3282 | -0.1933 | 0.2903 | 0.3585 | 0.2654 | 0.0197 | 0.0472 |
| Red skin % | 0.4494 | 0.6373 | 1.0000 | -0.0606 | 0.0551 | -0.1262 | -0.4678 | 0.2143 | 0.1099 | 0.1429 | 0.1288 | 0.1272 |
| TSS (°Bx) | -0.0105 | -0.3356 | -0.0606 | 1.0000 | 0.4767 | -0.2944 | -0.2158 | -0.5137 | -0.3507 | -0.4918 | -0.3767 | -0.3823 |
| Firmness (kg) | -0.1518 | -0.0140 | 0.0551 | 0.4767 | 1.0000 | 0.3010 | -0.2037 | -0.4404 | -0.2884 | -0.3956 | -0.3484 | -0.3475 |
| Yield (fruit) | -0.0140 | 0.3282 | -0.1262 | -0.2944 | 0.3010 | 1.0000 | -0.1586 | 0.0749 | 0.0769 | 0.0854 | 0.0139 | 0.0229 |
| Bitter pit | -0.0730 | -0.1933 | -0.4678 | -0.2158 | -0.2037 | -0.1586 | 1.0000 | -0.1559 | -0.0447 | -0.0818 | -0.2258 | -0.2232 |
| K/Ca | 0.3561 | 0.2903 | 0.2143 | -0.5137 | -0.4404 | 0.0749 | -0.1559 | 1.0000 | 0.7785 | 0.9283 | 0.6145 | 0.6353 |
| Mg/Ca | 0.5150 | 0.3585 | 0.1099 | -0.3507 | -0.2884 | 0.0769 | -0.0447 | 0.7785 | 1.0000 | 0.9166 | 0.2449 | 0.3212 |
| P/Ca | 0.4281 | 0.2654 | 0.1429 | -0.4918 | -0.3956 | 0.0854 | -0.0818 | 0.9283 | 0.9166 | 1.0000 | 0.5053 | 0.5552 |
| K+Mg/Ca | 0.1948 | 0.0197 | 0.1288 | -0.3767 | -0.3484 | 0.0139 | -0.2258 | 0.6145 | 0.2449 | 0.5053 | 1.0000 | 0.9948 |
| K+Mg+P/Ca | 0.2429 | 0.0472 | 0.1272 | -0.3823 | -0.3475 | 0.0229 | -0.2232 | 0.6353 | 0.3212 | 0.5552 | 0.9948 | 1.0000 |

## Multivariate correlations
## Rootstock=G202

Table 39.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from G.202 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2095 | -0.2832 | -0.4085 | -0.2355 | -0.0383 | 0.3298 | -0.0540 | -0.4743 | 0.6305 | -0.3546 |
| Fruit weight (g) | 0.2095 | 1.0000 | -0.1278 | 0.3395 | 0.2502 | 0.2046 | 0.1988 | -0.0892 | -0.1116 | 0.2073 | -0.1234 |
| Red skin % | -0.2832 | -0.1278 | 1.0000 | 0.5087 | -0.2924 | 0.2857 | -0.1103 | -0.1102 | 0.2945 | -0.2196 | 0.1691 |
| TSS (°Bx) | -0.4085 | 0.3395 | 0.5087 | 1.0000 | 0.3298 | 0.5255 | -0.2514 | -0.2423 | 0.2235 | -0.3544 | 0.1000 |
| Firmness (kg) | -0.2355 | 0.2502 | -0.2924 | 0.3298 | 1.0000 | 0.1165 | -0.1449 | -0.4338 | 0.0595 | -0.3016 | -0.2756 |
| Yield (fruit) | -0.0383 | 0.2046 | 0.2857 | 0.5255 | 0.1165 | 1.0000 | -0.4088 | -0.5131 | -0.2455 | -0.2218 | -0.2004 |
| P | 0.3298 | 0.1988 | -0.1103 | -0.2514 | -0.1449 | -0.4088 | 1.0000 | 0.6691 | 0.3394 | 0.6205 | 0.3598 |
| K | -0.0540 | -0.0892 | -0.1102 | -0.2423 | -0.4338 | -0.5131 | 0.6691 | 1.0000 | 0.3843 | 0.5114 | 0.6596 |
| Ca | -0.4743 | -0.1116 | 0.2945 | 0.2235 | 0.0595 | -0.2455 | 0.3394 | 0.3843 | 1.0000 | -0.3642 | 0.5843 |
| Mg | 0.6305 | 0.2073 | -0.2196 | -0.3544 | -0.3016 | -0.2218 | 0.6205 | 0.5114 | -0.3642 | 1.0000 | -0.1216 |
| S | -0.3546 | -0.1234 | 0.1691 | 0.1000 | -0.2756 | -0.2004 | 0.3598 | 0.6596 | 0.5843 | -0.1216 | 1.0000 |

## Rootstock=G202

Table 40.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from G.202 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2095 | -0.2832 | -0.4085 | -0.2355 | -0.0383 | -0.2605 | -0.2832 | 0.0677 | -0.6775 | -0.0319 |
| Fruit weight (g) | 0.2095 | 1.0000 | -0.1278 | 0.3395 | 0.2502 | 0.2046 | -0.3220 | -0.3971 | -0.0888 | -0.2093 | -0.1906 |
| Red skin % | -0.2832 | -0.1278 | 1.0000 | 0.5087 | -0.2924 | 0.2857 | -0.3215 | 0.0987 | 0.0863 | 0.1691 | -0.0559 |
| TSS (°Bx) | -0.4085 | 0.3395 | 0.5087 | 1.0000 | 0.3298 | 0.5255 | -0.2458 | 0.1564 | -0.0312 | 0.2609 | -0.0833 |
| Firmness (kg) | -0.2355 | 0.2502 | -0.2924 | 0.3298 | 1.0000 | 0.1165 | -0.0724 | -0.1854 | -0.2456 | -0.0322 | -0.1326 |
| Yield (fruit) | -0.0383 | 0.2046 | 0.2857 | 0.5255 | 0.1165 | 1.0000 | -0.2802 | 0.0545 | -0.1224 | -0.0176 | -0.1065 |
| B | -0.2605 | -0.3220 | -0.3215 | -0.2458 | -0.0724 | -0.2802 | 1.0000 | 0.6544 | 0.3148 | 0.6197 | 0.5398 |
| Zn | -0.2832 | -0.3971 | 0.0987 | 0.1564 | -0.1854 | 0.0545 | 0.6544 | 1.0000 | 0.5906 | 0.7542 | 0.4852 |
| Fe | 0.0677 | -0.0888 | 0.0863 | -0.0312 | -0.2456 | -0.1224 | 0.3148 | 0.5906 | 1.0000 | 0.3038 | 0.4562 |
| Mn | -0.6775 | -0.2093 | 0.1691 | 0.2609 | -0.0322 | -0.0176 | 0.6197 | 0.7542 | 0.3038 | 1.0000 | 0.1459 |
| Cu | -0.0319 | -0.1906 | -0.0559 | -0.0833 | -0.1326 | -0.1065 | 0.5398 | 0.4852 | 0.4562 | 0.1459 | 1.0000 |

## Rootstock=G202

Table 41.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from G.202 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2095 | -0.2832 | -0.4085 | -0.2355 | -0.0383 | 0.3414 | 0.4871 | 0.6299 | 0.6674 | -0.0536 | -0.0041 |
| Fruit weight (g) | 0.2095 | 1.0000 | -0.1278 | 0.3395 | 0.2502 | 0.2046 | 0.3243 | 0.2639 | 0.3696 | 0.4470 | -0.0889 | -0.0697 |
| Red skin % | -0.2832 | -0.1278 | 1.0000 | 0.5087 | -0.2924 | 0.2857 | -0.3309 | -0.4477 | -0.3811 | -0.4103 | -0.1104 | -0.1224 |
| TSS (°Bx) | -0.4085 | 0.3395 | 0.5087 | 1.0000 | 0.3298 | 0.5255 | 0.1578 | -0.3164 | -0.2666 | -0.3594 | -0.2425 | -0.2593 |
| Firmness (kg) | -0.2355 | 0.2502 | -0.2924 | 0.3298 | 1.0000 | 0.1165 | 0.1412 | -0.2717 | -0.1706 | -0.1933 | -0.4339 | -0.4389 |
| Yield (fruit) | -0.0383 | 0.2046 | 0.2857 | 0.5255 | 0.1165 | 1.0000 | -0.0087 | -0.0564 | 0.0511 | -0.1174 | -0.5131 | -0.5090 |
| Bitter pit | 0.3414 | 0.3243 | -0.3309 | 0.1578 | 0.1412 | -0.0087 | 1.0000 | 0.2925 | 0.2980 | 0.3321 | -0.1439 | -0.1438 |
| K/Ca | 0.4871 | 0.2639 | -0.4477 | -0.3164 | -0.2717 | -0.0564 | 0.2925 | 1.0000 | 0.9555 | 0.8693 | 0.1854 | 0.2300 |
| Mg/Ca | 0.6299 | 0.3696 | -0.3811 | -0.2666 | -0.1706 | 0.0511 | 0.2980 | 0.9555 | 1.0000 | 0.9055 | -0.0092 | 0.0449 |
| P/Ca | 0.6674 | 0.4470 | -0.4103 | -0.3594 | -0.1933 | -0.1174 | 0.3321 | 0.8693 | 0.9055 | 1.0000 | 0.2071 | 0.2596 |
| K+Mg/Ca | -0.0536 | -0.0889 | -0.1104 | -0.2425 | -0.4339 | -0.5131 | -0.1439 | 0.1854 | -0.0092 | 0.2071 | 1.0000 | 0.9979 |
| K+Mg+P/Ca | -0.0041 | -0.0697 | -0.1224 | -0.2593 | -0.4389 | -0.5090 | -0.1438 | 0.2300 | 0.0449 | 0.2596 | 0.9979 | 1.0000 |

## Multivariate correlations
## Rootstock=G. 214

Table 42.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from G.214 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.1941 | 0.1956 | -0.2736 | -0.2028 | -0.0176 | 0.2440 | 0.0211 | -0.2240 | 0.4974 | -0.3718 |
| Fruit weight (g) | 0.1941 | 1.0000 | 0.4117 | 0.1515 | -0.0516 | -0.1917 | 0.1949 | -0.6050 | -0.3318 | 0.6472 | -0.6250 |
| Red skin % | 0.1956 | 0.4117 | 1.0000 | -0.4441 | -0.3805 | -0.1076 | 0.1015 | -0.4869 | -0.6155 | 0.2370 | -0.2115 |
| TSS (°Bx) | -0.2736 | 0.1515 | -0.4441 | 1.0000 | 0.6213 | 0.3117 | -0.0237 | -0.2705 | 0.3034 | -0.0166 | 0.0321 |
| Firmness (kg) | -0.2028 | -0.0516 | -0.3805 | 0.6213 | 1.0000 | 0.3114 | 0.1225 | -0.0070 | 0.3293 | 0.2014 | 0.1639 |
| Yield (fruit) | -0.0176 | -0.1917 | -0.1076 | 0.3117 | 0.3114 | 1.0000 | -0.0576 | 0.2378 | 0.3057 | 0.0034 | 0.3474 |
| P | 0.2440 | 0.1949 | 0.1015 | -0.0237 | 0.1225 | -0.0576 | 1.0000 | 0.1661 | -0.2054 | 0.6878 | -0.3174 |
| K | 0.0211 | -0.6050 | -0.4869 | -0.2705 | -0.0070 | 0.2378 | 0.1661 | 1.0000 | 0.2273 | -0.1826 | 0.4667 |
| Ca | -0.2240 | -0.3318 | -0.6155 | 0.3034 | 0.3293 | 0.3057 | -0.2054 | 0.2273 | 1.0000 | -0.1127 | 0.0923 |
| Mg | 0.4974 | 0.6472 | 0.2370 | -0.0166 | 0.2014 | 0.0034 | 0.6878 | -0.1826 | -0.1127 | 1.0000 | -0.6167 |
| S | -0.3718 | -0.6250 | -0.2115 | 0.0321 | 0.1639 | 0.3474 | -0.3174 | 0.4667 | 0.0923 | -0.6167 | 1.0000 |

## Rootstock=G. 214

Table 43.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from G.214 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.1941 | 0.1956 | -0.2736 | -0.2028 | -0.0176 | -0.1866 | -0.0461 | -0.2099 | -0.2096 | -0.1576 |
| Fruit weight (g) | 0.1941 | 1.0000 | 0.4117 | 0.1515 | -0.0516 | -0.1917 | -0.1309 | 0.1546 | 0.0015 | -0.2934 | 0.4266 |
| Red skin % | 0.1956 | 0.4117 | 1.0000 | -0.4441 | -0.3805 | -0.1076 | 0.5575 | -0.1074 | -0.2115 | -0.4581 | 0.0321 |
| TSS (°Bx) | -0.2736 | 0.1515 | -0.4441 | 1.0000 | 0.6213 | 0.3117 | -0.4973 | 0.5156 | 0.2484 | 0.1351 | 0.1203 |
| Firmness (kg) | -0.2028 | -0.0516 | -0.3805 | 0.6213 | 1.0000 | 0.3114 | -0.2500 | 0.6458 | 0.3256 | 0.1631 | 0.2452 |
| Yield (fruit) | -0.0176 | -0.1917 | -0.1076 | 0.3117 | 0.3114 | 1.0000 | -0.1584 | 0.6062 | 0.3133 | 0.1511 | 0.0025 |
| B | -0.1866 | -0.1309 | 0.5575 | -0.4973 | -0.2500 | -0.1584 | 1.0000 | -0.2831 | -0.0138 | -0.1811 | 0.0011 |
| Zn | -0.0461 | 0.1546 | -0.1074 | 0.5156 | 0.6458 | 0.6062 | -0.2831 | 1.0000 | 0.2274 | 0.2868 | 0.4127 |
| Fe | -0.2099 | 0.0015 | -0.2115 | 0.2484 | 0.3256 | 0.3133 | -0.0138 | 0.2274 | 1.0000 | 0.4442 | 0.1521 |
| Mn | -0.2096 | -0.2934 | -0.4581 | 0.1351 | 0.1631 | 0.1511 | -0.1811 | 0.2868 | 0.4442 | 1.0000 | 0.0989 |
| Cu | -0.1576 | 0.4266 | 0.0321 | 0.1203 | 0.2452 | 0.0025 | 0.0011 | 0.4127 | 0.1521 | 0.0989 | 1.0000 |

## Rootstock=G. 214

Table 44.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from G.214 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.1941 | 0.1956 | -0.2736 | -0.2028 | -0.0176 | 0.6161 | 0.2612 | 0.5672 | 0.3488 | 0.0214 | 0.0840 |
| Fruit weight (g) | 0.1941 | 1.0000 | 0.4117 | 0.1515 | -0.0516 | -0.1917 | 0.1661 | -0.0662 | 0.6382 | 0.2912 | -0.6048 | -0.5329 |
| Red skin % | 0.1956 | 0.4117 | 1.0000 | -0.4441 | -0.3805 | -0.1076 | 0.1378 | 0.3470 | 0.5493 | 0.4967 | -0.4867 | -0.4644 |
| TSS (°Bx) | -0.2736 | 0.1515 | -0.4441 | 1.0000 | 0.6213 | 0.3117 | -0.0616 | -0.4413 | -0.1943 | -0.2064 | -0.2706 | -0.2768 |
| Firmness (kg) | -0.2028 | -0.0516 | -0.3805 | 0.6213 | 1.0000 | 0.3114 | -0.1653 | -0.3569 | -0.0847 | -0.1840 | -0.0070 | 0.0181 |
| Yield (fruit) | -0.0176 | -0.1917 | -0.1076 | 0.3117 | 0.3114 | 1.0000 | 0.0913 | -0.2335 | -0.2078 | -0.2556 | 0.2377 | 0.2417 |
| Bitter pit | 0.6161 | 0.1661 | 0.1378 | -0.0616 | -0.1653 | 0.0913 | 1.0000 | 0.1759 | 0.4097 | 0.4062 | -0.0427 | -0.0016 |
| K/Ca | 0.2612 | -0.0662 | 0.3470 | -0.4413 | -0.3569 | -0.2335 | 0.1759 | 1.0000 | 0.5156 | 0.7510 | 0.2899 | 0.2948 |
| Mg/Ca | 0.5672 | 0.6382 | 0.5493 | -0.1943 | -0.0847 | -0.2078 | 0.4097 | 0.5156 | 1.0000 | 0.8364 | -0.3020 | -0.2076 |
| P/Ca | 0.3488 | 0.2912 | 0.4967 | -0.2064 | -0.1840 | -0.2556 | 0.4062 | 0.7510 | 0.8364 | 1.0000 | -0.1019 | -0.0441 |
| K+Mg/Ca | 0.0214 | -0.6048 | -0.4867 | -0.2706 | -0.0070 | 0.2377 | -0.0427 | 0.2899 | -0.3020 | -0.1019 | 1.0000 | 0.9924 |
| K+Mg+P/Ca | 0.0840 | -0.5329 | -0.4644 | -0.2768 | 0.0181 | 0.2417 | -0.0016 | 0.2948 | -0.2076 | -0.0441 | 0.9924 | 1.0000 |

## Multivariate correlations
## Rootstock=G935

Table 45.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from G.935 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.1382 | 0.0315 | -0.3741 | -0.1036 | -0.0921 | 0.7598 | 0.0389 | -0.4788 | 0.7390 | -0.7397 |
| Fruit weight (g) | 0.1382 | 1.0000 | 0.2866 | -0.5509 | -0.3032 | 0.0791 | 0.4397 | -0.1824 | -0.5420 | 0.5600 | -0.6250 |
| Red skin % | 0.0315 | 0.2866 | 1.0000 | 0.2283 | -0.0870 | 0.1596 | 0.2075 | 0.5245 | -0.2843 | 0.2060 | -0.0960 |
| TSS (°Bx) | -0.3741 | -0.5509 | 0.2283 | 1.0000 | 0.2272 | 0.1033 | -0.3181 | 0.4337 | 0.0703 | -0.4048 | 0.4046 |
| Firmness (kg) | -0.1036 | -0.3032 | -0.0870 | 0.2272 | 1.0000 | 0.2650 | -0.1633 | 0.2757 | -0.0903 | -0.5588 | 0.3704 |
| Yield (fruit) | -0.0921 | 0.0791 | 0.1596 | 0.1033 | 0.2650 | 1.0000 | 0.3026 | 0.2987 | -0.1141 | 0.0644 | 0.0848 |
| P | 0.7598 | 0.4397 | 0.2075 | -0.3181 | -0.1633 | 0.3026 | 1.0000 | 0.1324 | -0.5878 | 0.7560 | -0.5755 |
| K | 0.0389 | -0.1824 | 0.5245 | 0.4337 | 0.2757 | 0.2987 | 0.1324 | 1.0000 | 0.0849 | -0.0407 | 0.2696 |
| Ca | -0.4788 | -0.5420 | -0.2843 | 0.0703 | -0.0903 | -0.1141 | -0.5878 | 0.0849 | 1.0000 | -0.3752 | 0.5090 |
| Mg | 0.7390 | 0.5600 | 0.2060 | -0.4048 | -0.5588 | 0.0644 | 0.7560 | -0.0407 | -0.3752 | 1.0000 | -0.7851 |
| S | -0.7397 | -0.6250 | -0.0960 | 0.4046 | 0.3704 | 0.0848 | -0.5755 | 0.2696 | 0.5090 | -0.7851 | 1.0000 |

## Rootstock=G935

Table 46.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from G.935 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.1382 | 0.0315 | -0.3741 | -0.1036 | -0.0921 | -0.2858 | -0.0582 | -0.1588 | -0.6340 | 0.3545 |
| Fruit weight (g) | 0.1382 | 1.0000 | 0.2866 | -0.5509 | -0.3032 | 0.0791 | -0.0500 | -0.5030 | -0.2270 | -0.6887 | 0.1497 |
| Red skin % | 0.0315 | 0.2866 | 1.0000 | 0.2283 | -0.0870 | 0.1596 | 0.2172 | -0.3006 | 0.0250 | -0.2676 | 0.1415 |
| TSS (°Bx) | -0.3741 | -0.5509 | 0.2283 | 1.0000 | 0.2272 | 0.1033 | 0.1925 | 0.2954 | 0.2673 | 0.3385 | -0.2648 |
| Firmness (kg) | -0.1036 | -0.3032 | -0.0870 | 0.2272 | 1.0000 | 0.2650 | 0.1580 | 0.3234 | -0.3704 | 0.0213 | -0.2055 |
| Yield (fruit) | -0.0921 | 0.0791 | 0.1596 | 0.1033 | 0.2650 | 1.0000 | 0.0434 | -0.0581 | -0.0893 | -0.0432 | 0.0170 |
| B | -0.2858 | -0.0500 | 0.2172 | 0.1925 | 0.1580 | 0.0434 | 1.0000 | 0.2102 | 0.3946 | 0.1149 | -0.0585 |
| Zn | -0.0582 | -0.5030 | -0.3006 | 0.2954 | 0.3234 | -0.0581 | 0.2102 | 1.0000 | 0.3560 | 0.5686 | 0.1327 |
| Fe | -0.1588 | -0.2270 | 0.0250 | 0.2673 | -0.3704 | -0.0893 | 0.3946 | 0.3560 | 1.0000 | 0.3890 | 0.2837 |
| Mn | -0.6340 | -0.6887 | -0.2676 | 0.3385 | 0.0213 | -0.0432 | 0.1149 | 0.5686 | 0.3890 | 1.0000 | -0.0254 |
| Cu | 0.3545 | 0.1497 | 0.1415 | -0.2648 | -0.2055 | 0.0170 | -0.0585 | 0.1327 | 0.2837 | -0.0254 | 1.0000 |

## Rootstock=G935

Table 47.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from G.935 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.1382 | 0.0315 | -0.3741 | -0.1036 | -0.0921 | 0.2189 | 0.4238 | 0.6706 | 0.6401 | 0.0394 | 0.1436 |
| Fruit weight (g) | 0.1382 | 1.0000 | 0.2866 | -0.5509 | -0.3032 | 0.0791 | -0.0227 | 0.4011 | 0.6118 | 0.5057 | -0.1820 | -0.1005 |
| Red skin % | 0.0315 | 0.2866 | 1.0000 | 0.2283 | -0.0870 | 0.1596 | 0.0663 | 0.5118 | 0.3126 | 0.3113 | 0.5248 | 0.5532 |
| TSS (°Bx) | -0.3741 | -0.5509 | 0.2283 | 1.0000 | 0.2272 | 0.1033 | 0.0167 | 0.0576 | -0.3073 | -0.2576 | 0.4335 | 0.3745 |
| Firmness (kg) | -0.1036 | -0.3032 | -0.0870 | 0.2272 | 1.0000 | 0.2650 | -0.2746 | 0.1385 | -0.2606 | -0.0861 | 0.2755 | 0.1957 |
| Yield (fruit) | -0.0921 | 0.0791 | 0.1596 | 0.1033 | 0.2650 | 1.0000 | -0.0944 | 0.1959 | 0.0550 | 0.1790 | 0.2987 | 0.3067 |
| Bitter pit | 0.2189 | -0.0227 | 0.0663 | 0.0167 | -0.2746 | -0.0944 | 1.0000 | 0.1428 | 0.2118 | 0.1836 | -0.0853 | -0.0611 |
| K/Ca | 0.4238 | 0.4011 | 0.5118 | 0.0576 | 0.1385 | 0.1959 | 0.1428 | 1.0000 | 0.8262 | 0.8864 | 0.2092 | 0.2594 |
| Mg/Ca | 0.6706 | 0.6118 | 0.3126 | -0.3073 | -0.2606 | 0.0550 | 0.2118 | 0.8262 | 1.0000 | 0.9477 | -0.1385 | -0.0308 |
| P/Ca | 0.6401 | 0.5057 | 0.3113 | -0.2576 | -0.0861 | 0.1790 | 0.1836 | 0.8864 | 0.9477 | 1.0000 | -0.0280 | 0.0601 |
| K+Mg/Ca | 0.0394 | -0.1820 | 0.5248 | 0.4335 | 0.2755 | 0.2987 | -0.0853 | 0.2092 | -0.1385 | -0.0280 | 1.0000 | 0.9900 |
| K+Mg+P/Ca | 0.1436 | -0.1005 | 0.5532 | 0.3745 | 0.1957 | 0.3067 | -0.0611 | 0.2594 | -0.0308 | 0.0601 | 0.9900 | 1.0000 |

## Multivariate correlations
## Rootstock=M9

Table 48.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from M.9 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.3488 | -0.1484 | -0.5065 | -0.5668 | -0.5270 | 0.5851 | 0.1319 | -0.4970 | 0.5128 | -0.4754 |
| Fruit weight (g) | 0.3488 | 1.0000 | 0.1526 | -0.1010 | -0.3277 | -0.3961 | 0.2422 | -0.0965 | 0.2169 | 0.5257 | -0.1367 |
| Red skin % | -0.1484 | 0.1526 | 1.0000 | 0.0781 | 0.3993 | -0.0149 | -0.1638 | -0.7051 | 0.2157 | -0.1451 | -0.2072 |
| TSS (°Bx) | -0.5065 | -0.1010 | 0.0781 | 1.0000 | 0.4914 | 0.1586 | -0.2211 | 0.1069 | 0.0145 | -0.3169 | 0.7992 |
| Firmness (kg) | -0.5668 | -0.3277 | 0.3993 | 0.4914 | 1.0000 | 0.1904 | -0.5007 | -0.2983 | 0.0210 | -0.5316 | 0.3156 |
| Yield (fruit) | -0.5270 | -0.3961 | -0.0149 | 0.1586 | 0.1904 | 1.0000 | -0.3355 | -0.1890 | 0.1643 | -0.4313 | -0.0007 |
| P | 0.5851 | 0.2422 | -0.1638 | -0.2211 | -0.5007 | -0.3355 | 1.0000 | 0.3199 | -0.3026 | 0.6212 | -0.0656 |
| K | 0.1319 | -0.0965 | -0.7051 | 0.1069 | -0.2983 | -0.1890 | 0.3199 | 1.0000 | -0.2291 | 0.0718 | 0.4396 |
| Ca | -0.4970 | 0.2169 | 0.2157 | 0.0145 | 0.0210 | 0.1643 | -0.3026 | -0.2291 | 1.0000 | -0.2289 | 0.0138 |
| Mg | 0.5128 | 0.5257 | -0.1451 | -0.3169 | -0.5316 | -0.4313 | 0.6212 | 0.0718 | -0.2289 | 1.0000 | -0.2255 |
| S | -0.4754 | -0.1367 | -0.2072 | 0.7992 | 0.3156 | -0.0007 | -0.0656 | 0.4396 | 0.0138 | -0.2255 | 1.0000 |

## Rootstock=M9

Table 49.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from M.9 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.3488 | -0.1484 | -0.5065 | -0.5668 | -0.5270 | -0.2412 | -0.1009 | -0.1561 | -0.6736 | 0.4726 |
| Fruit weight (g) | 0.3488 | 1.0000 | 0.1526 | -0.1010 | -0.3277 | -0.3961 | 0.0792 | 0.0229 | 0.4411 | 0.0696 | 0.2056 |
| Red skin % | -0.1484 | 0.1526 | 1.0000 | 0.0781 | 0.3993 | -0.0149 | 0.0400 | -0.0491 | 0.4396 | -0.3164 | -0.2820 |
| TSS (°Bx) | -0.5065 | -0.1010 | 0.0781 | 1.0000 | 0.4914 | 0.1586 | 0.2420 | -0.0219 | -0.3043 | 0.4745 | -0.2928 |
| Firmness (kg) | -0.5668 | -0.3277 | 0.3993 | 0.4914 | 1.0000 | 0.1904 | -0.1816 | 0.3769 | -0.0665 | 0.1722 | -0.5535 |
| Yield (fruit) | -0.5270 | -0.3961 | -0.0149 | 0.1586 | 0.1904 | 1.0000 | 0.2343 | -0.2000 | 0.0428 | 0.0968 | -0.3116 |
| B | -0.2412 | 0.0792 | 0.0400 | 0.2420 | -0.1816 | 0.2343 | 1.0000 | -0.0610 | 0.3807 | 0.1185 | 0.0890 |
| Zn | -0.1009 | 0.0229 | -0.0491 | -0.0219 | 0.3769 | -0.2000 | -0.0610 | 1.0000 | 0.1245 | 0.2307 | 0.0242 |
| Fe | -0.1561 | 0.4411 | 0.4396 | -0.3043 | -0.0665 | 0.0428 | 0.3807 | 0.1245 | 1.0000 | 0.0782 | -0.0563 |
| Mn | -0.6736 | 0.0696 | -0.3164 | 0.4745 | 0.1722 | 0.0968 | 0.1185 | 0.2307 | 0.0782 | 1.0000 | -0.2639 |
| Cu | 0.4726 | 0.2056 | -0.2820 | -0.2928 | -0.5535 | -0.3116 | 0.0890 | 0.0242 | -0.0563 | -0.2639 | 1.0000 |

## Rootstock=M9

Table 50.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from M.9 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.3488 | -0.1484 | -0.5065 | -0.5668 | -0.5270 | 0.2893 | 0.4626 | 0.6681 | 0.6857 | 0.1322 | 0.1802 |
| Fruit weight (g) | 0.3488 | 1.0000 | 0.1526 | -0.1010 | -0.3277 | -0.3961 | -0.0761 | -0.2317 | 0.1552 | 0.0487 | -0.0964 | -0.0403 |
| Red skin % | -0.1484 | 0.1526 | 1.0000 | 0.0781 | 0.3993 | -0.0149 | -0.0732 | -0.4094 | -0.2099 | -0.2152 | -0.7052 | -0.7071 |
| TSS (°Bx) | -0.5065 | -0.1010 | 0.0781 | 1.0000 | 0.4914 | 0.1586 | -0.2502 | -0.0290 | -0.2408 | -0.2121 | 0.1067 | 0.0749 |
| Firmness (kg) | -0.5668 | -0.3277 | 0.3993 | 0.4914 | 1.0000 | 0.1904 | -0.3945 | -0.1226 | -0.3341 | -0.3705 | -0.2985 | -0.3462 |
| Yield (fruit) | -0.5270 | -0.3961 | -0.0149 | 0.1586 | 0.1904 | 1.0000 | 0.2749 | -0.2459 | -0.4381 | -0.4005 | -0.1892 | -0.2286 |
| Bitter pit | 0.2893 | -0.0761 | -0.0732 | -0.2502 | -0.3945 | 0.2749 | 1.0000 | -0.2261 | 0.0162 | 0.1707 | -0.2850 | -0.2652 |
| K/Ca | 0.4626 | -0.2317 | -0.4094 | -0.0290 | -0.1226 | -0.2459 | -0.2261 | 1.0000 | 0.7639 | 0.7623 | 0.5740 | 0.5912 |
| Mg/Ca | 0.6681 | 0.1552 | -0.2099 | -0.2408 | -0.3341 | -0.4381 | 0.0162 | 0.7639 | 1.0000 | 0.8401 | 0.1842 | 0.2543 |
| P/Ca | 0.6857 | 0.0487 | -0.2152 | -0.2121 | -0.3705 | -0.4005 | 0.1707 | 0.7623 | 0.8401 | 1.0000 | 0.3434 | 0.3970 |
| K+Mg/ca | 0.1322 | -0.0964 | -0.7052 | 0.1067 | -0.2985 | -0.1892 | -0.2850 | 0.5740 | 0.1842 | 0.3434 | 1.0000 | 0.9954 |
| K+Mg+P/Ca | 0.1802 | -0.0403 | -0.7071 | 0.0749 | -0.3462 | -0.2286 | -0.2652 | 0.5912 | 0.2543 | 0.3970 | 0.9954 | 1.0000 |

## Multivariate correlations
## Rootstock=M26

Table 51.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder and peel nutrients of Honeycrisp' from M26 rootstocks at Ithaca from 2018.

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | P | K | Ca | Mg | S |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.3022 | -0.0432 | -0.6960 | -0.1027 | -0.0191 | 0.1467 | -0.0814 | -0.1576 | 0.2013 | -0.5177 |
| Fruit weight (g) | 0.3022 | 1.0000 | 0.2553 | -0.2822 | 0.2155 | -0.0009 | -0.1163 | -0.0967 | -0.0753 | 0.1581 | -0.5512 |
| Red skin % | -0.0432 | 0.2553 | 1.0000 | 0.0733 | 0.0573 | 0.0704 | -0.0717 | -0.0665 | -0.2577 | 0.0627 | -0.3044 |
| TSS (°Bx) | -0.6960 | -0.2822 | 0.0733 | 1.0000 | 0.1863 | -0.3088 | -0.2307 | 0.0084 | 0.0577 | -0.3007 | 0.5018 |
| Firmness (kg) | -0.1027 | 0.2155 | 0.0573 | 0.1863 | 1.0000 | 0.0384 | 0.1732 | 0.2001 | 0.1265 | 0.0550 | -0.0347 |
| Yield (fruit) | -0.0191 | -0.0009 | 0.0704 | -0.3088 | 0.0384 | 1.0000 | 0.1008 | -0.0771 | -0.1815 | 0.0360 | 0.0287 |
| P | 0.1467 | -0.1163 | -0.0717 | -0.2307 | 0.1732 | 0.1008 | 1.0000 | 0.7498 | 0.1631 | 0.7729 | 0.2836 |
| K | -0.0814 | -0.0967 | -0.0665 | 0.0084 | 0.2001 | -0.0771 | 0.7498 | 1.0000 | 0.2081 | 0.5682 | 0.3554 |
| Ca | -0.1576 | -0.0753 | -0.2577 | 0.0577 | 0.1265 | -0.1815 | 0.1631 | 0.2081 | 1.0000 | 0.0358 | 0.1608 |
| Mg | 0.2013 | 0.1581 | 0.0627 | -0.3007 | 0.0550 | 0.0360 | 0.7729 | 0.5682 | 0.0358 | 1.0000 | -0.0907 |
| S | -0.5177 | -0.5512 | -0.3044 | 0.5018 | -0.0347 | 0.0287 | 0.2836 | 0.3554 | 0.1608 | -0.0907 | 1.0000 |

## Rootstock=M26

Table 52.Multivarite correlation between soil pH and fruit maturity parameters, and peel nutrients of Honeycrisp' from M.26 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | B | Zn | Fe | Mn | Cu |
|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.3022 | -0.0432 | -0.6960 | -0.1027 | -0.0191 | -0.4939 | -0.1280 | 0.1171 | -0.6989 | 0.1009 |
| Fruit weight (g) | 0.3022 | 1.0000 | 0.2553 | -0.2822 | 0.2155 | -0.0009 | -0.2013 | -0.1188 | 0.1762 | -0.3988 | 0.1025 |
| Red skin % | -0.0432 | 0.2553 | 1.0000 | 0.0733 | 0.0573 | 0.0704 | 0.0065 | -0.1164 | 0.1397 | -0.0528 | 0.0410 |
| TSS (°Bx) | -0.6960 | -0.2822 | 0.0733 | 1.0000 | 0.1863 | -0.3088 | 0.4334 | 0.0082 | 0.0197 | 0.6404 | -0.1917 |
| Firmness (kg) | -0.1027 | 0.2155 | 0.0573 | 0.1863 | 1.0000 | 0.0384 | 0.0156 | 0.1464 | 0.3870 | 0.0149 | -0.0340 |
| Yield (fruit) | -0.0191 | -0.0009 | 0.0704 | -0.3088 | 0.0384 | 1.0000 | 0.0040 | -0.0434 | 0.1419 | 0.0819 | 0.2627 |
| B | -0.4939 | -0.2013 | 0.0065 | 0.4334 | 0.0156 | 0.0040 | 1.0000 | 0.4255 | 0.0415 | 0.5774 | 0.3359 |
| Zn | -0.1280 | -0.1188 | -0.1164 | 0.0082 | 0.1464 | -0.0434 | 0.4255 | 1.0000 | 0.1073 | 0.1794 | 0.5425 |
| Fe | 0.1171 | 0.1762 | 0.1397 | 0.0197 | 0.3870 | 0.1419 | 0.0415 | 0.1073 | 1.0000 | 0.0925 | 0.3270 |
| Mn | -0.6989 | -0.3988 | -0.0528 | 0.6404 | 0.0149 | 0.0819 | 0.5774 | 0.1794 | 0.0925 | 1.0000 | -0.0948 |
| Cu | 0.1009 | 0.1025 | 0.0410 | -0.1917 | -0.0340 | 0.2627 | 0.3359 | 0.5425 | 0.3270 | -0.0948 | 1.0000 |

## Rootstock=M26

Table 53.Multivarite correlation between soil pH and fruit maturity parameters, storage disorder, and peel nutrients ratio of Honeycrisp' from M.26 rootstocks at Ithaca from 2018

| | pH | Fruit weight (g) | Red skin % | TSS (°Bx) | Firmness (kg) | Yield (fruit) | Bitter pit | K/Ca | Mg/Ca | P/Ca | K+Mg/Ca | K+Mg+P/Ca |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.3022 | -0.0432 | -0.6960 | -0.1027 | -0.0191 | -0.0165 | 0.1121 | 0.2899 | 0.2259 | -0.0812 | -0.0652 |
| Fruit weight (g) | 0.3022 | 1.0000 | 0.2553 | -0.2822 | 0.2155 | -0.0009 | -0.0419 | 0.0164 | 0.1683 | -0.0213 | -0.0966 | -0.0828 |
| Red skin % | -0.0432 | 0.2553 | 1.0000 | 0.0733 | 0.0573 | 0.0704 | -0.0335 | 0.1715 | 0.2452 | 0.1211 | -0.0664 | -0.0598 |
| TSS (°Bx) | -0.6960 | -0.2822 | 0.0733 | 1.0000 | 0.1863 | -0.3088 | 0.2444 | -0.1040 | -0.2734 | -0.2777 | 0.0083 | -0.0114 |
| Firmness (kg) | -0.1027 | 0.2155 | 0.0573 | 0.1863 | 1.0000 | 0.0384 | -0.1758 | -0.0215 | -0.1112 | 0.0390 | 0.2001 | 0.1960 |
| Yield (fruit) | -0.0191 | -0.0009 | 0.0704 | -0.3088 | 0.0384 | 1.0000 | -0.2114 | 0.1485 | 0.2070 | 0.2620 | -0.0770 | -0.0717 |
| Bitter pit | -0.0165 | -0.0419 | -0.0335 | 0.2444 | -0.1758 | -0.2114 | 1.0000 | -0.0074 | 0.0224 | -0.1068 | -0.1427 | -0.1443 |
| K/Ca | 0.1121 | 0.0164 | 0.1715 | -0.1040 | -0.0215 | 0.1485 | -0.0074 | 1.0000 | 0.8372 | 0.8463 | 0.5358 | 0.5439 |
| Mg/Ca | 0.2899 | 0.1683 | 0.2452 | -0.2734 | -0.1112 | 0.2070 | 0.0224 | 0.8372 | 1.0000 | 0.8426 | 0.1630 | 0.1939 |
| P/Ca | 0.2259 | -0.0213 | 0.1211 | -0.2777 | 0.0390 | 0.2620 | -0.1068 | 0.8463 | 0.8426 | 1.0000 | 0.4800 | 0.5038 |
| K+Mg/Ca | -0.0812 | -0.0966 | -0.0664 | 0.0083 | 0.2001 | -0.0770 | -0.1427 | 0.5358 | 0.1630 | 0.4800 | 1.0000 | 0.9986 |
| K+Mg+P/Ca | -0.0652 | -0.0828 | -0.0598 | -0.0114 | 0.1960 | -0.0717 | -0.1443 | 0.5439 | 0.1939 | 0.5038 | 0.9986 | 1.0000 |

**Rootstock=B.9**
**Scatterplot Matrix**

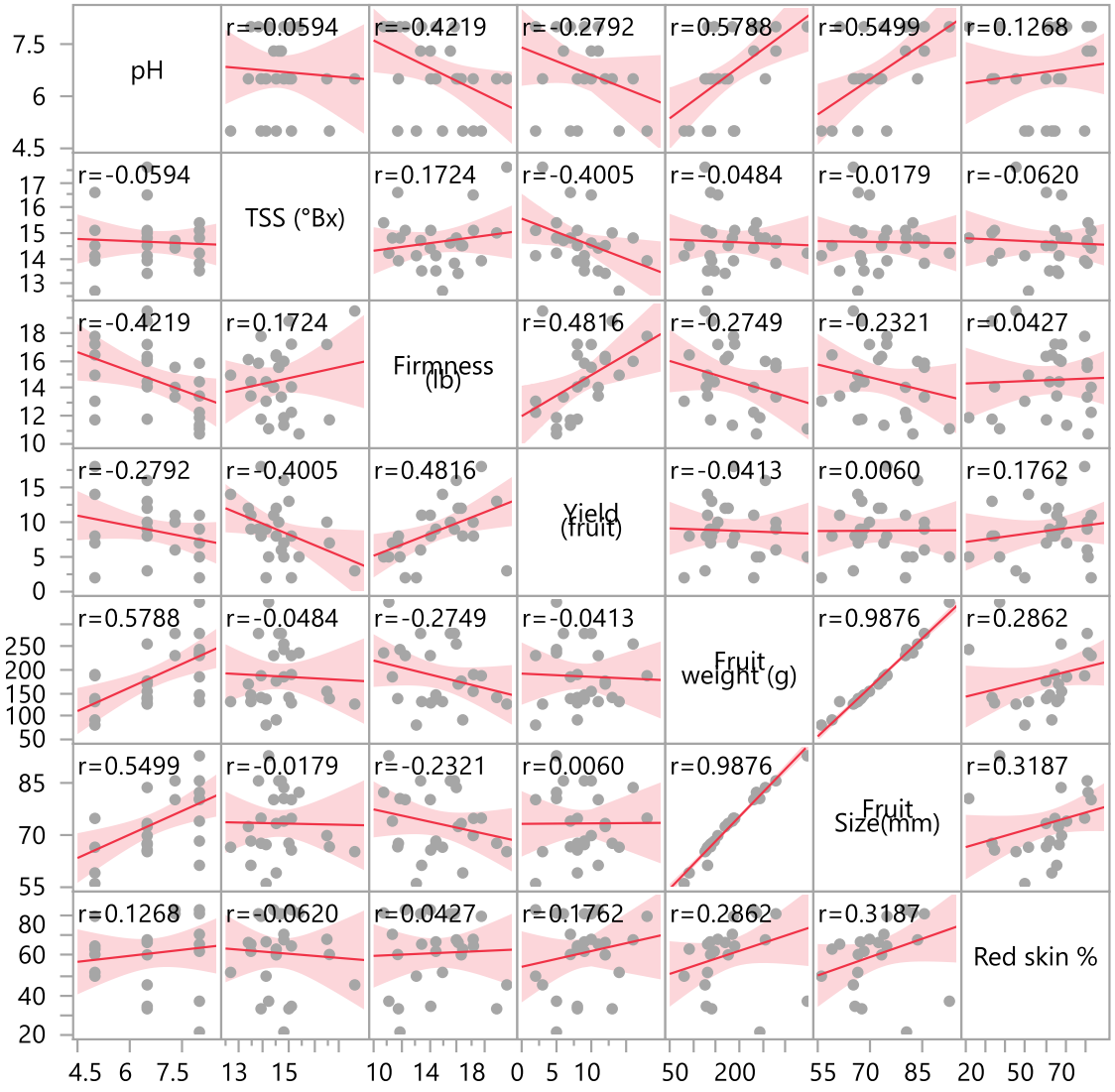


Figure 30.A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on.B.9 rootstock under varying soil pH levels at Ithaca

**Rootstdock=G.11**
**Scatterplot Matrix**

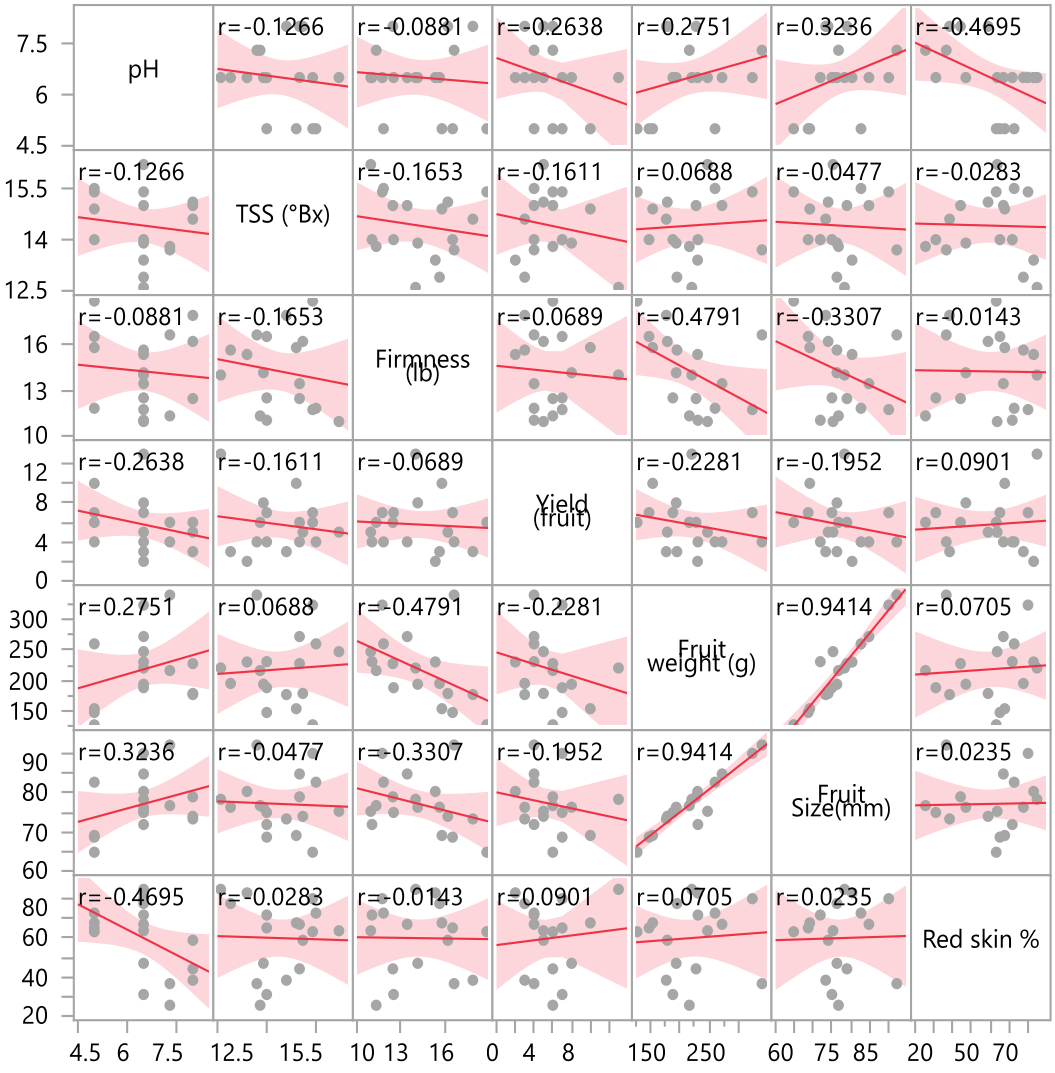


Figure 31.A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on G.11 rootstock under varying soil pH levels at Ithaca.

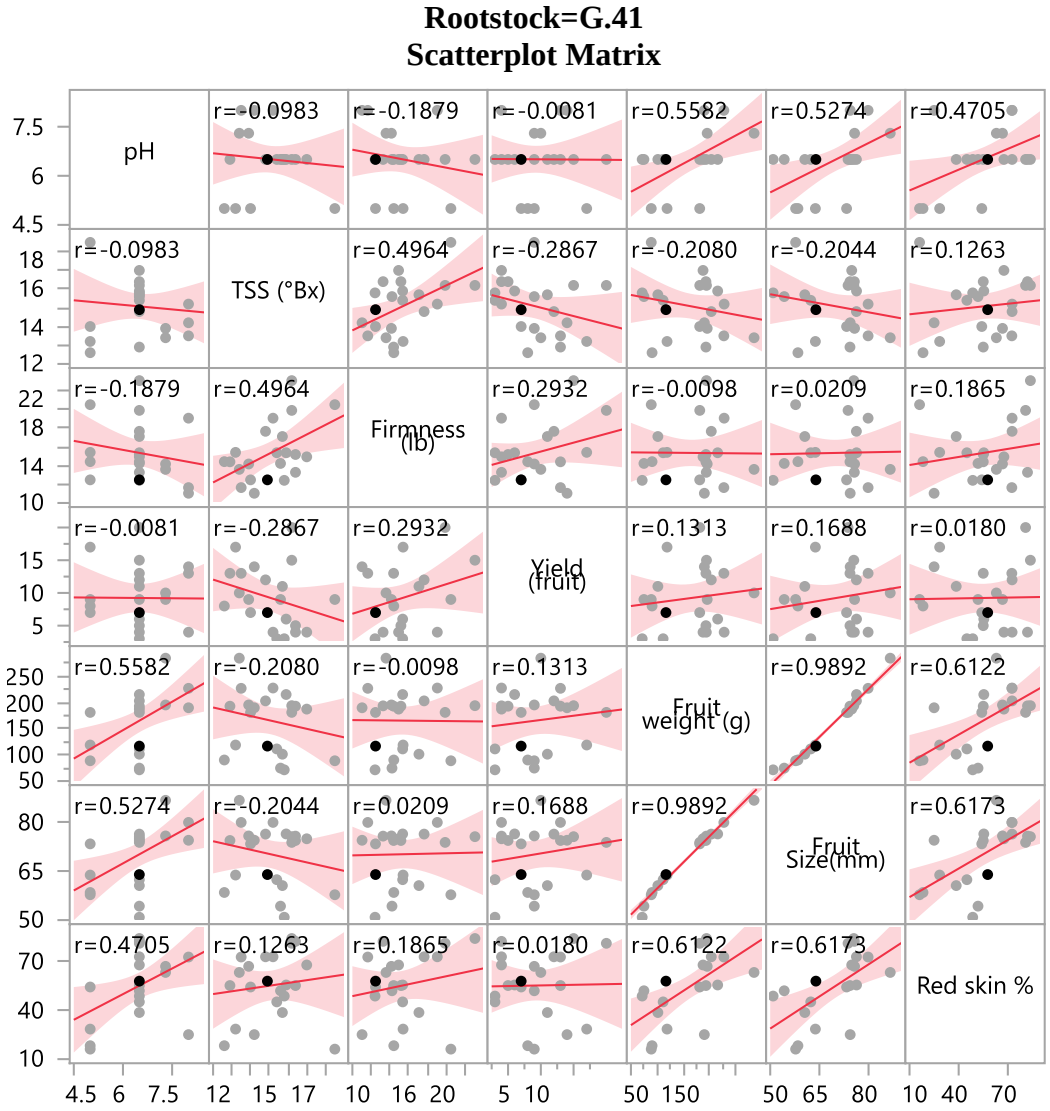


Figure 32. A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on G.41 rootstock under varying soil pH levels at Ithaca

**Rootstock=M.202**
**Scatterplot Matrix**

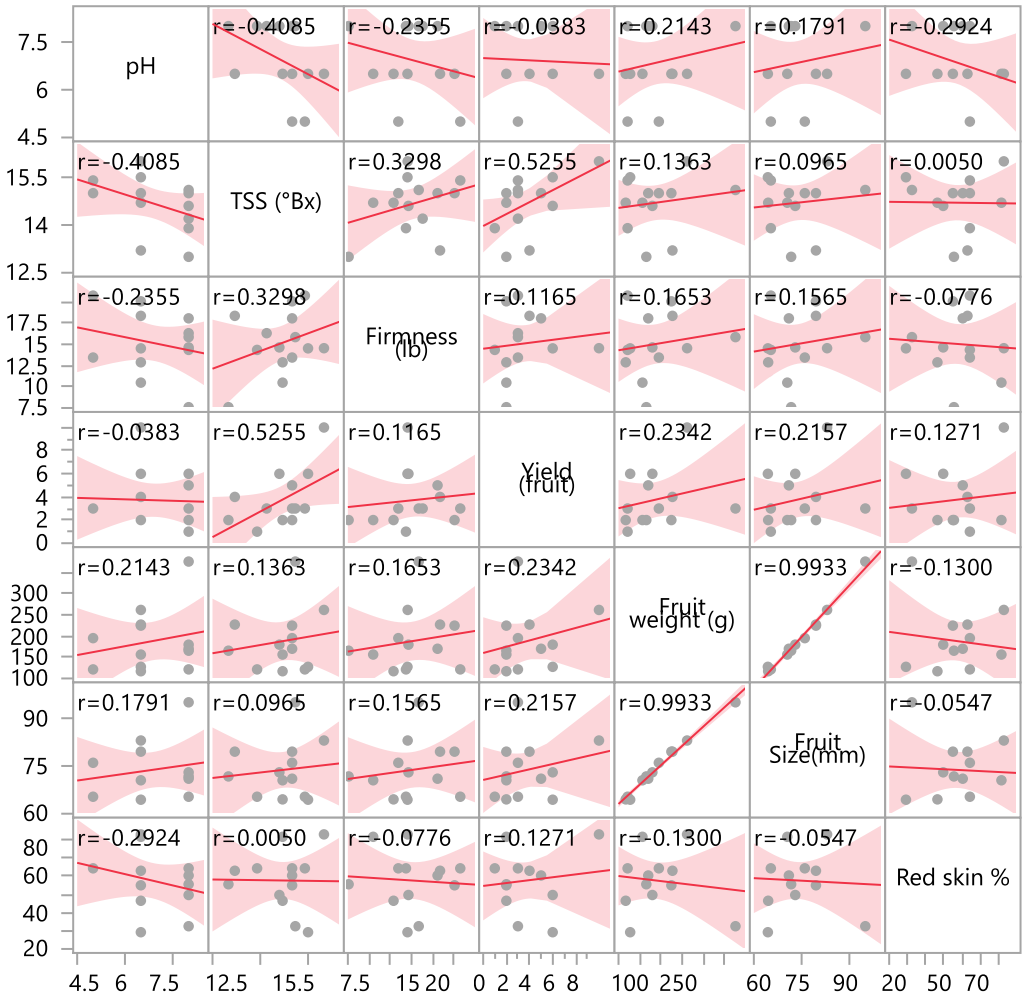


Figure 33 A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on G.202 rootstock under varying soil pH levels at *Ithaca.*

**Rootstock=G214**
**Scatterplot Matrix**

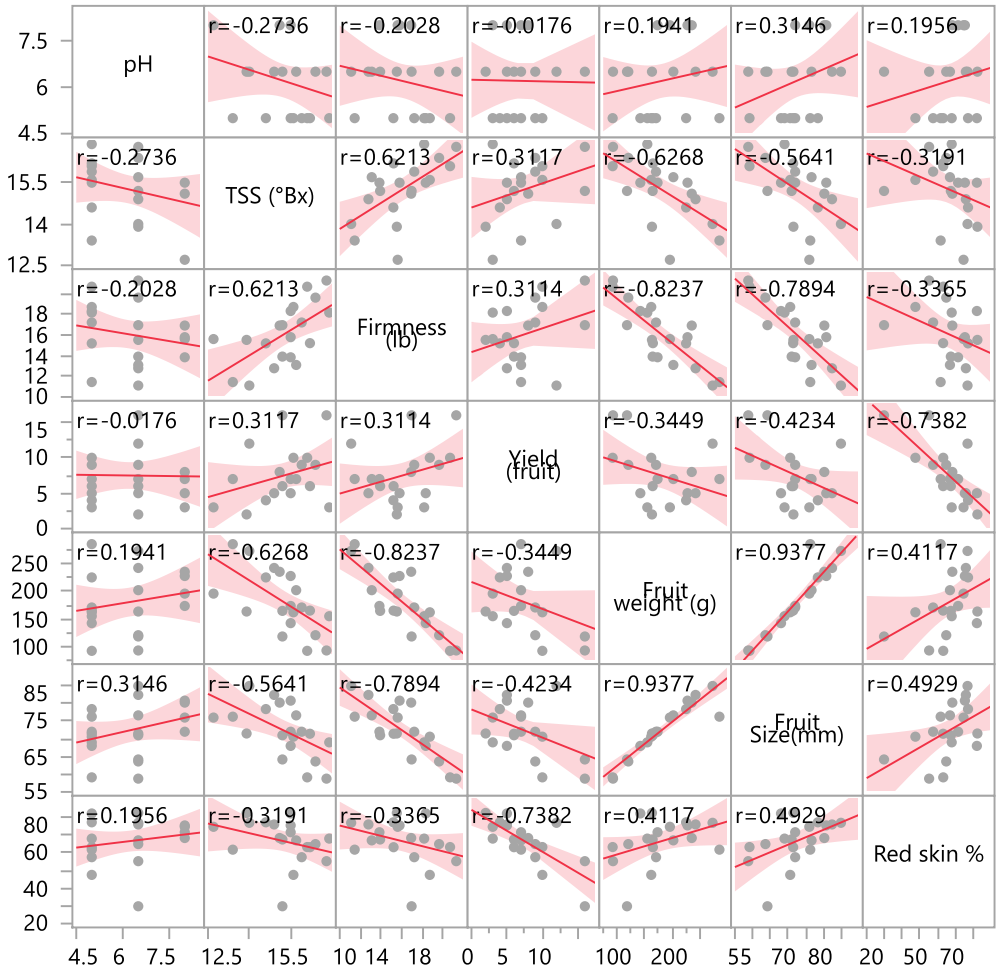


Figure 34.A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on G.214 rootstock under varying soil pH levels at *Ithaca*.

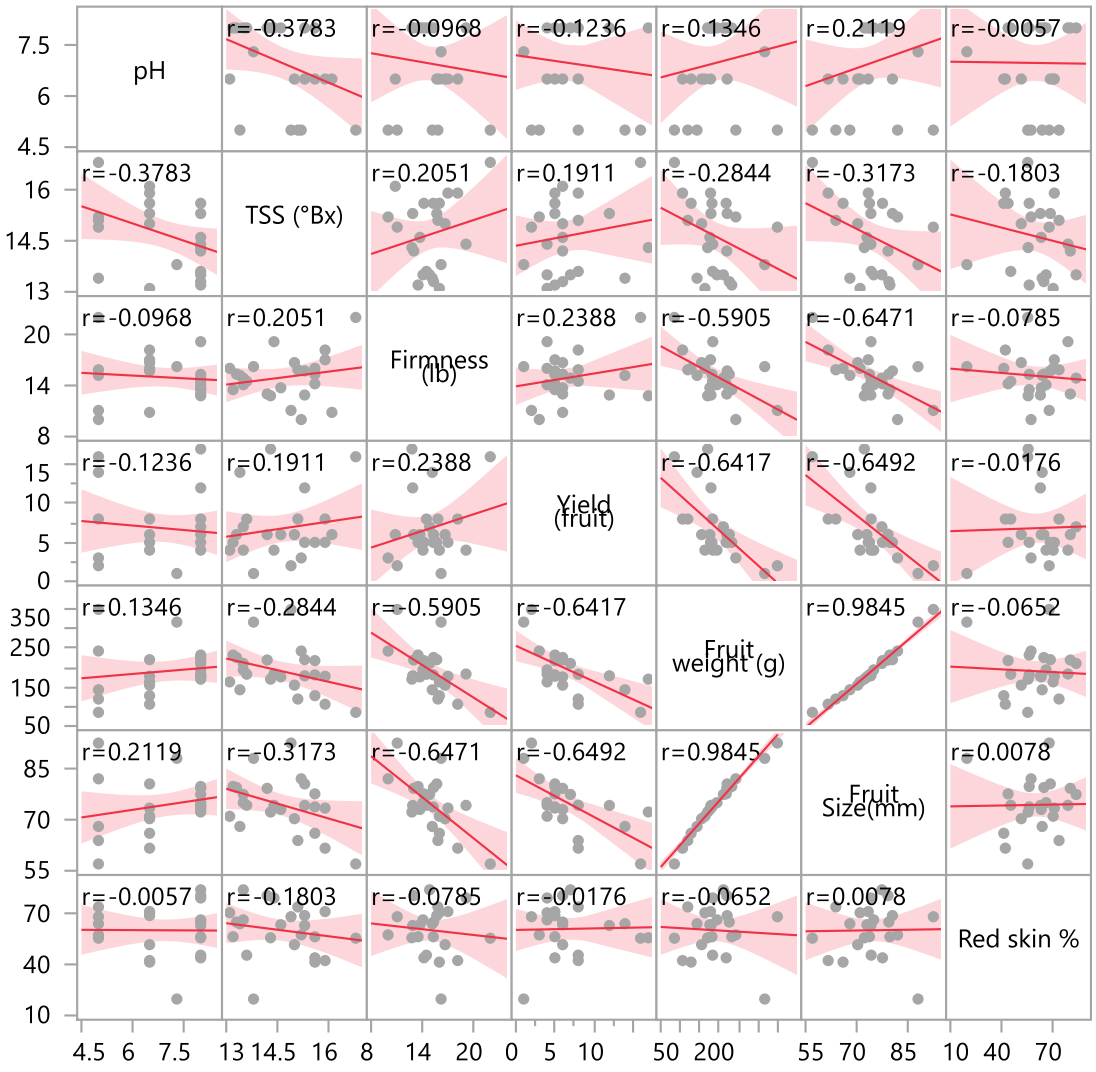


Figure 35.A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on G.935 rootstock under varying soil pH levels at Ithaca, NY.

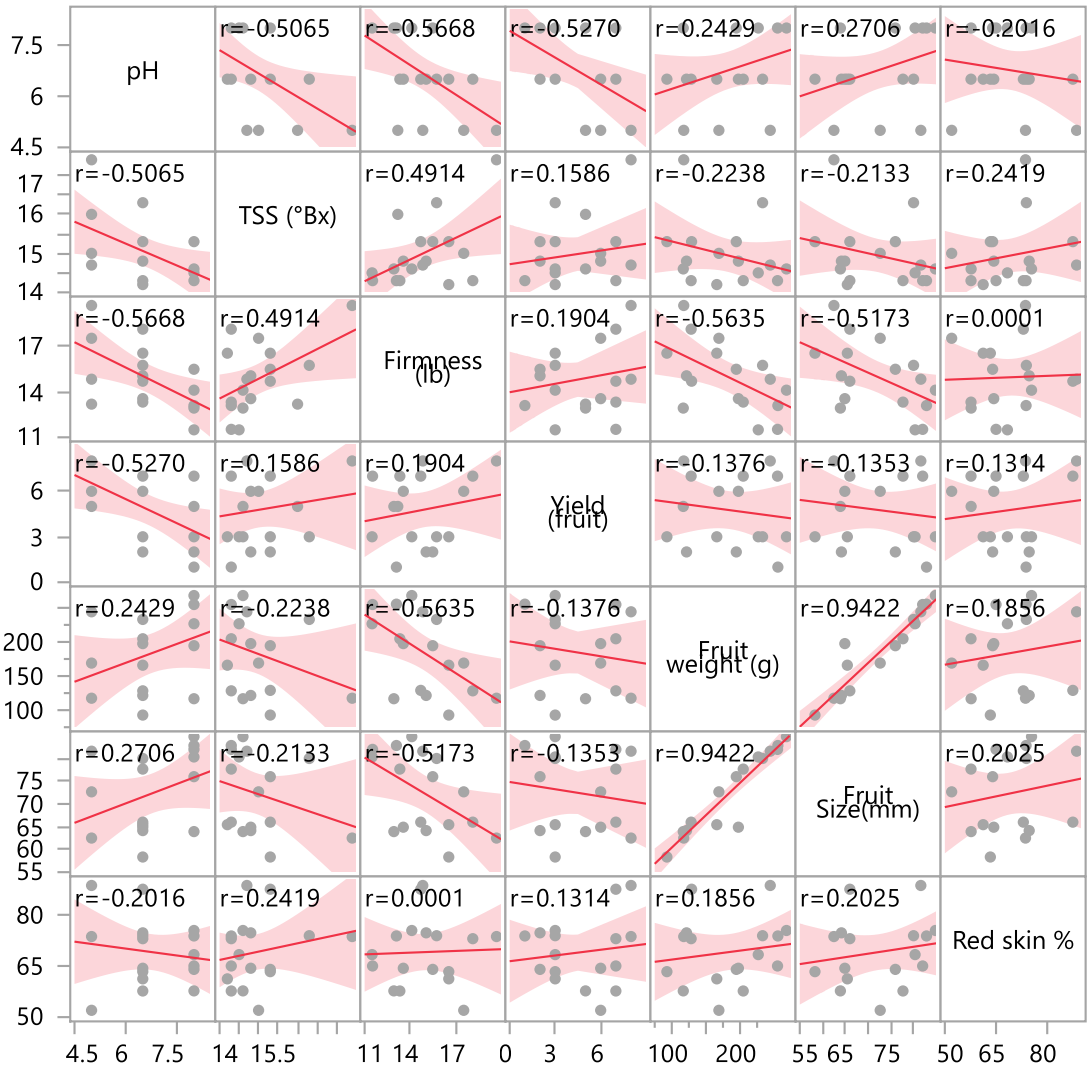


Figure 36.A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on M.9 rootstock under varying soil pH levels at Ithaca, *NY*.

**Rootstock=M.26**
**Scatterplot Matrix**

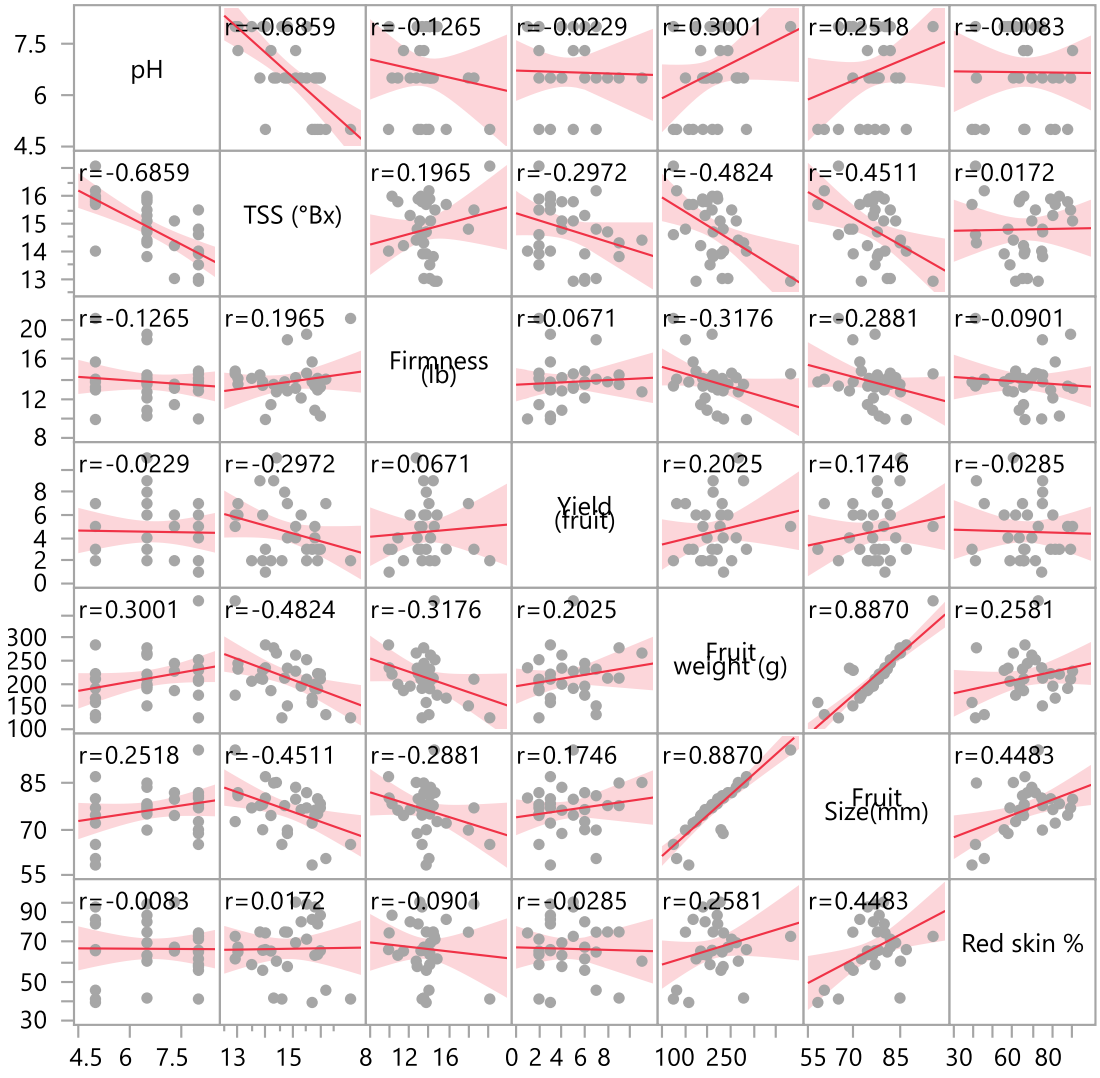


Figure 37. A multivariate scatterplot of means of fruit maturity parameters TSS, fruit firmness, yield, fruit's weight fruit's size and fruit red skin % from 'Honeycrisp' grown on M.26 rootstock under varying soil pH levels at Ithaca, NY.

## Multivariate Correlations
## Rootstock=G41

Table 54.Multivarite correlation between solution pH and root architecture parameters in cm (group 1& 2) G.41 rootstocks grown in aeroponics system at Geneva 2018.

| | pH | Average Root Width (Diameter) | Number of Connected Components | Maximum Number of Roots | Network Depth | Network Width | Network Area | Network Perimeter |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | -0.3515 | 0.3576 | 0.3095 | 0.1964 | 0.3735 | 0.2339 | 0.3224 |
| Average Root Width (Diameter) | -0.3515 | 1.0000 | -0.4559 | -0.5332 | -0.1413 | -0.3811 | -0.3237 | -0.4856 |
| Number of Connected Components | 0.3576 | -0.4559 | 1.0000 | 0.8232 | 0.7755 | 0.8316 | 0.8670 | 0.9149 |
| Maximum Number of Roots | 0.3095 | -0.5332 | 0.8232 | 1.0000 | 0.6168 | 0.7168 | 0.8876 | 0.9563 |
| Network Depth | 0.1964 | -0.1413 | 0.7755 | 0.6168 | 1.0000 | 0.8312 | 0.8724 | 0.7869 |
| Network Width | 0.3735 | -0.3811 | 0.8316 | 0.7168 | 0.8312 | 1.0000 | 0.8470 | 0.8264 |
| Network Area | 0.2339 | -0.3237 | 0.8670 | 0.8876 | 0.8724 | 0.8470 | 1.0000 | 0.9640 |
| Network Perimeter | 0.3224 | -0.4856 | 0.9149 | 0.9563 | 0.7869 | 0.8264 | 0.9640 | 1.0000 |

Table 55. Multivariate correlation between solution pH and root architecture parameters in cm (group 3) G.41 rootstocks grown in aeroponics system at Geneva 2018

| | pH | Network Surface Area | Network Length | Network Volume | Network Convex Area | Network Solidity | Network Bushiness | Network Length Distribution |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2396 | 0.3295 | 0.0858 | 0.4150 | -0.4594 | 0.0293 | -0.2192 |
| Network Surface Area | 0.2396 | 1.0000 | 0.9637 | 0.9246 | 0.8860 | -0.4876 | -0.3108 | -0.4172 |
| Network Length | 0.3295 | 0.9637 | 1.0000 | 0.7924 | 0.8827 | -0.5043 | -0.2314 | -0.4263 |
| Network Volume | 0.0858 | 0.9246 | 0.7924 | 1.0000 | 0.7734 | -0.4275 | -0.3753 | -0.3563 |
| Network Convex Area | 0.4150 | 0.8860 | 0.8827 | 0.7734 | 1.0000 | -0.7144 | -0.1601 | -0.4380 |
| Network Solidity | -0.4594 | -0.4876 | -0.5043 | -0.4275 | -0.7144 | 1.0000 | -0.1865 | 0.2101 |
| Network Bushiness | 0.0293 | -0.3108 | -0.2314 | -0.3753 | -0.1601 | -0.1865 | 1.0000 | 0.3541 |
| Network Length Distribution | -0.2192 | -0.4172 | -0.4263 | -0.3563 | -0.4380 | 0.2101 | 0.3541 | 1.0000 |

## Multivariate Correlations
## Rootstock=G210

Table 56. Multivarite correlation between solution pH and root architecture parameters in cm (group 1& 2) G.210 rootstocks grown in aeroponics system at Geneva 2018.

| | pH | Average Root Width (Diameter) | Number of Connected Components | Maximum Number of Roots | Network Depth | Network Width | Network Area | Network Perimeter |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | -0.1838 | 0.4329 | 0.2408 | 0.3020 | 0.4740 | 0.4132 | 0.3988 |
| Average Root Width (Diameter) | -0.1838 | 1.0000 | -0.2724 | -0.4356 | 0.0733 | -0.0512 | -0.1411 | -0.3042 |
| Number of Connected Components | 0.4329 | -0.2724 | 1.0000 | 0.6180 | 0.8304 | 0.9017 | 0.9331 | 0.9255 |
| Maximum Number of Roots | 0.2408 | -0.4356 | 0.6180 | 1.0000 | 0.5210 | 0.6002 | 0.7295 | 0.8330 |
| Network Depth | 0.3020 | 0.0733 | 0.8304 | 0.5210 | 1.0000 | 0.8343 | 0.8842 | 0.8306 |
| Network Width | 0.4740 | -0.0512 | 0.9017 | 0.6002 | 0.8343 | 1.0000 | 0.9513 | 0.8891 |
| Network Area | 0.4132 | -0.1411 | 0.9331 | 0.7295 | 0.8842 | 0.9513 | 1.0000 | 0.9736 |
| Network Perimeter | 0.3988 | -0.3042 | 0.9255 | 0.8330 | 0.8306 | 0.8891 | 0.9736 | 1.0000 |

Table 57.Multivariate correlation between solution pH and root architecture parameters in cm (group 3) G.210 rootstocks grown in aeroponics system at Geneva 2018

| | pH | Network Surface Area | Network Length | Network Volume | Network Convex Area | Network Solidity | Network Bushiness | Network Length Distribution |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.4174 | 0.4071 | 0.4202 | 0.4955 | -0.4398 | -0.1580 | -0.1775 |
| Network Surface Area | 0.4174 | 1.0000 | 0.9713 | 0.9786 | 0.9590 | -0.5015 | -0.1677 | -0.2951 |
| Network Length | 0.4071 | 0.9713 | 1.0000 | 0.9035 | 0.9090 | -0.4356 | -0.1985 | -0.2989 |
| Network Volume | 0.4202 | 0.9786 | 0.9035 | 1.0000 | 0.9593 | -0.5533 | -0.1202 | -0.2831 |
| Network Convex Area | 0.4955 | 0.9590 | 0.9090 | 0.9593 | 1.0000 | -0.5969 | -0.1319 | -0.3043 |
| Network Solidity | -0.4398 | -0.5015 | -0.4356 | -0.5533 | -0.5969 | 1.0000 | -0.2671 | -0.0048 |
| Network Bushiness | -0.1580 | -0.1677 | -0.1985 | -0.1202 | -0.1319 | -0.2671 | 1.0000 | 0.2942 |
| Network Length Distribution | -0.1775 | -0.2951 | -0.2989 | -0.2831 | -0.3043 | -0.0048 | 0.2942 | 1.0000 |

## Multivariate Correlations
## Rootstock=G214

Table 58.Multivariate correlation between solution pH and root architecture parameters in cm (group 1& 2) G.214 rootstocks grown in aeroponics system at Geneva 2018.

| | pH | Average Root Width (Diameter) | Number of Connected Components | Maximum Number of Roots | Network Depth | Network Width | Network Area | Network Perimeter |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | -0.0243 | 0.4273 | 0.1594 | 0.1801 | 0.4625 | 0.2817 | 0.2713 |
| Average Root Width (Diameter) | -0.0243 | 1.0000 | -0.3760 | -0.5664 | -0.0701 | -0.2169 | -0.2376 | -0.4438 |
| Number of Connected Components | 0.4273 | -0.3760 | 1.0000 | 0.6412 | 0.7285 | 0.8572 | 0.8295 | 0.8291 |
| Maximum Number of Roots | 0.1594 | -0.5664 | 0.6412 | 1.0000 | 0.5619 | 0.6393 | 0.8040 | 0.9027 |
| Network Depth | 0.1801 | -0.0701 | 0.7285 | 0.5619 | 1.0000 | 0.7192 | 0.8748 | 0.8124 |
| Network Width | 0.4625 | -0.2169 | 0.8572 | 0.6393 | 0.7192 | 1.0000 | 0.8604 | 0.8209 |
| Network Area | 0.2817 | -0.2376 | 0.8295 | 0.8040 | 0.8748 | 0.8604 | 1.0000 | 0.9520 |
| Network Perimeter | 0.2713 | -0.4438 | 0.8291 | 0.9027 | 0.8124 | 0.8209 | 0.9520 | 1.0000 |

Table 59.Multivarite correlation between solution pH and root architecture parameters in cm (group 3) G.214 rootstocks grown in aeroponics system at Geneva 2018

| | pH | Network Surface Area | Network Length | Network Volume | Network Convex Area | Network Solidity | Network Bushiness | Network Length Distribution |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2768 | 0.2680 | 0.2745 | 0.4559 | -0.4285 | 0.1982 | -0.1845 |
| Network Surface Area | 0.2768 | 1.0000 | 0.9501 | 0.9372 | 0.8994 | -0.3531 | -0.3347 | -0.2499 |
| Network Length | 0.2680 | 0.9501 | 1.0000 | 0.7845 | 0.8645 | -0.3503 | -0.3758 | -0.2626 |
| Network Volume | 0.2745 | 0.9372 | 0.7845 | 1.0000 | 0.8330 | -0.3393 | -0.2147 | -0.2074 |
| Network Convex Area | 0.4559 | 0.8994 | 0.8645 | 0.8330 | 1.0000 | -0.5930 | -0.2324 | -0.3055 |
| Network Solidity | -0.4285 | -0.3531 | -0.3503 | -0.3393 | -0.5930 | 1.0000 | -0.2846 | 0.0456 |
| Network Bushiness | 0.1982 | -0.3347 | -0.3758 | -0.2147 | -0.2324 | -0.2846 | 1.0000 | 0.4846 |
| Network Length Distribution | -0.1845 | -0.2499 | -0.2626 | -0.2074 | -0.3055 | 0.0456 | 0.4846 | 1.0000 |

## Multivariate Correlations
## Rootstock=G890

Table 60. Multivarite correlation between solution pH and root architecture parameters in cm (group 1& 2) G.890 rootstocks grown in aeroponics system at Geneva 2018.

| | pH | Average Root Width (Diameter) | Number of Connected Components | Maximum Number of Roots | Network Depth | Network Width | Network Area | Network Perimeter |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | -0.1521 | 0.3771 | 0.1522 | 0.1479 | 0.4114 | 0.2216 | 0.1883 |
| Average Root Width (Diameter) | -0.1521 | 1.0000 | -0.1526 | -0.4505 | 0.2051 | -0.0582 | -0.1054 | -0.3169 |
| Number of Connected Components | 0.3771 | -0.1526 | 1.0000 | 0.6148 | 0.7694 | 0.9195 | 0.8178 | 0.7546 |
| Maximum Number of Roots | 0.1522 | -0.4505 | 0.6148 | 1.0000 | 0.5129 | 0.5780 | 0.8385 | 0.9507 |
| Network Depth | 0.1479 | 0.2051 | 0.7694 | 0.5129 | 1.0000 | 0.7963 | 0.8327 | 0.6989 |
| Network Width | 0.4114 | -0.0582 | 0.9195 | 0.5780 | 0.7963 | 1.0000 | 0.8491 | 0.7365 |
| Network Area | 0.2216 | -0.1054 | 0.8178 | 0.8385 | 0.8327 | 0.8491 | 1.0000 | 0.9486 |
| Network Perimeter | 0.1883 | -0.3169 | 0.7546 | 0.9507 | 0.6989 | 0.7365 | 0.9486 | 1.0000 |

Table 61. Multivarite correlation between solution pH and root architecture parameters in cm (group 3) G.890 rootstocks grown in aeroponics system at Geneva 2018.

| | pH | Network Surface Area | Network Length | Network Volume | Network Convex Area | Network Solidity | Network Bushiness | Network Length Distribution |
|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.2231 | 0.2020 | 0.2284 | 0.4102 | -0.2891 | 0.1071 | -0.4274 |
| Network Surface Area | 0.2231 | 1.0000 | 0.9523 | 0.9344 | 0.8509 | -0.2520 | -0.1444 | -0.2839 |
| Network Length | 0.2020 | 0.9523 | 1.0000 | 0.7836 | 0.7621 | -0.1543 | -0.1892 | -0.3212 |
| Network Volume | 0.2284 | 0.9344 | 0.7836 | 1.0000 | 0.8568 | -0.3678 | -0.0531 | -0.1971 |
| Network Convex Area | 0.4102 | 0.8509 | 0.7621 | 0.8568 | 1.0000 | -0.5852 | -0.0742 | -0.2843 |
| Network Solidity | -0.2891 | -0.2520 | -0.1543 | -0.3678 | -0.5852 | 1.0000 | -0.2476 | -0.0362 |
| Network Bushiness | 0.1071 | -0.1444 | -0.1892 | -0.0531 | -0.0742 | -0.2476 | 1.0000 | 0.2800 |
| Network Length Distribution | -0.4274 | -0.2839 | -0.3212 | -0.1971 | -0.2843 | -0.0362 | 0.2800 | 1.0000 |

Table 62.Multivariate correlation between soil pH and leaf nutrients of Honeycrisp apple on four rootstocks grown in minirhizotron system 2018.

| | pH | N | P | K | Ca | Mg | S | B | Zn | Cu | Mn | Na | Fe |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| pH | 1.0000 | 0.0209 | 0.3479 | 0.6048 | 0.6048 | 0.4748 | -0.3162 | 0.3821 | 0.0477 | -0.0440 | 0.3479 | -0.3106 | -0.3498 |
| N | 0.0209 | 1.0000 | 0.8061 | 0.3557 | 0.3557 | 0.4382 | 0.7740 | -0.2137 | 0.2416 | 0.2625 | 0.4296 | -0.2133 | 0.2564 |
| P | 0.3479 | 0.8061 | 1.0000 | 0.5441 | 0.5441 | 0.5532 | 0.5357 | 0.0451 | 0.3099 | 0.2753 | 0.4110 | -0.2728 | 0.1394 |
| K | 0.6048 | 0.3557 | 0.5441 | 1.0000 | 1.0000 | 0.7129 | 0.1566 | 0.3389 | 0.3298 | 0.1346 | 0.6037 | 0.1127 | 0.1992 |
| Ca | 0.6048 | 0.3557 | 0.5441 | 1.0000 | 1.0000 | 0.7129 | 0.1566 | 0.3389 | 0.3298 | 0.1346 | 0.6037 | 0.1127 | 0.1992 |
| Mg | 0.4748 | 0.4382 | 0.5532 | 0.7129 | 0.7129 | 1.0000 | 0.1997 | -0.0747 | 0.2978 | 0.0340 | 0.3022 | -0.1856 | 0.2683 |
| S | -0.3162 | 0.7740 | 0.5357 | 0.1566 | 0.1566 | 0.1997 | 1.0000 | -0.3065 | 0.3362 | 0.2806 | 0.1822 | 0.1351 | 0.4608 |
| B | 0.3821 | -0.2137 | 0.0451 | 0.3389 | 0.3389 | -0.0747 | -0.3065 | 1.0000 | 0.1268 | 0.1715 | 0.1492 | -0.0785 | -0.1518 |
| Zn | 0.0477 | 0.2416 | 0.3099 | 0.3298 | 0.3298 | 0.2978 | 0.3362 | 0.1268 | 1.0000 | 0.4368 | 0.2455 | 0.0784 | 0.5191 |
| Cu | -0.0440 | 0.2625 | 0.2753 | 0.1346 | 0.1346 | 0.0340 | 0.2806 | 0.1715 | 0.4368 | 1.0000 | 0.1501 | -0.0940 | 0.2008 |
| Mn | 0.3479 | 0.4296 | 0.4110 | 0.6037 | 0.6037 | 0.3022 | 0.1822 | 0.1492 | 0.2455 | 0.1501 | 1.0000 | 0.1142 | 0.1105 |
| Na | -0.3106 | -0.2133 | -0.2728 | 0.1127 | 0.1127 | -0.1856 | 0.1351 | -0.0785 | 0.0784 | -0.0940 | 0.1142 | 1.0000 | 0.3613 |
| Fe | -0.3498 | 0.2564 | 0.1394 | 0.1992 | 0.1992 | 0.2683 | 0.4608 | -0.1518 | 0.5191 | 0.2008 | 0.1105 | 0.3613 | 1.0000 |

Table 63.Multivariate correlation between soil pH and root morphology parameters of Honeycrisp' from four rootstocks grown in minirhizotron system 2017-2018

| | Depth | pH | DAP | Sum (Root Count) | Sum (Total Root Length) | Sum (Total Root Volume ^3) | Sum (Total Root Area ^2) | Mean (Average Root Diameter) | Mean (Average Root Area ^2) | Mean (Average Root Volume ^3) | Mean (Average Root Length) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Depth | 1.0000 | -0.0819 | 0.2289 | -0.0455 | -0.0519 | -0.0517 | -0.0730 | -0.2377 | -0.0517 | -0.0517 | -0.1195 |
| pH | -0.0819 | 1.0000 | -0.0238 | -0.2574 | -0.2720 | -0.0580 | -0.0329 | 0.0076 | -0.0580 | -0.0580 | -0.0146 |
| DAP | 0.2289 | -0.0238 | 1.0000 | 0.1608 | 0.0907 | -0.0141 | -0.0753 | -0.5412 | -0.0141 | -0.0141 | -0.3839 |
| Sum (Root Count) | -0.0455 | -0.2574 | 0.1608 | 1.0000 | 0.9791 | -0.0353 | -0.0238 | -0.3274 | -0.0353 | -0.0353 | -0.2461 |
| Sum (Total Root Length) | -0.0519 | -0.2720 | 0.0907 | 0.9791 | 1.0000 | 0.1565 | 0.0221 | -0.2874 | -0.2465 | -0.1905 | -0.1542 |
| Sum (Total Root Volume ^3) | -0.0517 | -0.0580 | -0.0141 | -0.0353 | 0.1565 | 1.0000 | 0.6471 | 0.5088 | 1.0000 | 1.0000 | 0.2913 |
| Sum (Total Root Area ^2) | -0.0730 | -0.0329 | -0.0753 | -0.0238 | 0.0221 | 0.6471 | 1.0000 | 0.0077 | 0.6471 | 0.6471 | 0.0413 |
| Mean(Average Root Diameter) | -0.2377 | 0.0076 | -0.5412 | -0.3274 | -0.2874 | 0.5088 | 0.0077 | 1.0000 | 0.8747 | 0.8778 | 0.4026 |
| Mean(Average Root Length) | -0.1195 | -0.0146 | -0.3839 | -0.2461 | -0.1542 | 0.2913 | 0.0413 | 0.4026 | 0.7069 | 0.5519 | 1.0000 |
| Mean(Average Root Area ^2) | -0.0517 | -0.0580 | -0.0141 | -0.0353 | -0.2465 | 1.0000 | 0.6471 | 0.8747 | 1.0000 | 1.0000 | 0.7069 |
| Mean(Average Root Volume ^3) | -0.0517 | -0.0580 | -0.0141 | -0.0353 | -0.1905 | 1.0000 | 0.6471 | 0.8778 | 1.0000 | 1.0000 | 0.5519 |